University of Alberta

Bioremediation Of Hydrocarbon Contaminated And Compost Amended Soil: Effects On Microbial Communities In The Field

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment

of the requirements for the degree of

Master of Science

in

Land Reclamation and Remediation

Department of Renewable Resources

Edmonton, Alberta

Fall 2005

0-494-09183-5

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ABSTRACT

Microbial degradation of hydrocarbons in contaminated soil is improved by compost application, making compost a potential amendment for bioremediation of recalcitrant CCME fraction 3 and 4 ($>C_{16}$) hydrocarbons. Compost application increases soil organic matter content, nutrient status and soil aeration, thus stimulating microbial populations and in turn improving soil structure.

The effects of compost amendment, with and without fertiliser additions, on microbial communities associated with crude oil bioremediation was studied in a simulated in situ field trial in Edmonton, Alberta over 14 months. With 20% dw compost application, aerobic heterotrophic and aliphatic hydrocarbon-degrading microorganisms, microbial biomass carbon, aerobic respiration rate and microbial community diversity and richness were greatly increased as hydrocarbons decreased. Ammonium sulphate fertiliser suppressed microbial community growth and activity by acidifying soil, but this effect was buffered by compost co-application. Aliphatic hydrocarbon-degrading microoganism population size, microbial biomass carbon and aerobic respiration rate were positively correlated with petroleum hydrocarbon degradation.

ACKNOWLEDGEMENTS

Thanks to the City of Edmonton and the Department of Renewable Resources for funding and supporting this project. I am especially grateful to my supervisors, Dr. M. Anne Naeth and Dr. Allen (Al) Jobson, and to my family for their support and guidance. Thank you to everyone who helped to get this work done:

Dr. Julia M. Foght
Ms. Iris Bitterlich
Mr. Thomas (Tom) Hantos
Ms. Sarah Wilkinson
Ms. Suzanne (Sue) Graham
Ms. Chung Nguyen
Mr. Dean Mackenzie
Ms. Denise Harmon
Mr. Kory Jackson
Mr. Benjamin (Ben) Zhao
Mr. Joseph (Joe) Zhao
Ms. Bonnie Kwiatkowski
Dr. Salim Abboud
Ms. Frances De Jong
Mr. Gregory (Greg) Lewin

Dedicated to the memory of Alvin and Pauline Watkinson

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1. BIOREMEDIATION OF HYDROCARBON CONTAMINATED SOIL: AN OVERVIEW

1.1 INTRODUCTION

Contamination of soil from petroleum hydrocarbons is widespread in western Canada, with an estimated 75,000 to 90,000 contaminated sites (McIntyre 2003). Leaking fuel storage tanks, spills at petroleum wellsites, ruptured pipelines and spills at refuelling stations are among the main sources of natural gas, diesel fuel or crude oil contamination in Alberta (Frick et al. 1999; Salanitro 2001; McIntyre 2002; Rahman et al. 2002; Germida and Farrell 2003).

Natural attenuation and bioremediation have proven to be effective remediation technologies for sites with low to moderate levels of hydrocarbon contamination and suitable soil and climate conditions. These treatments work by allowing native or inoculated soil microorganisms to degrade the organic contaminants over time (Lye et al. 1997; Riser-Roberts 1998; Frick et al. 1999; Stanley et al. 2000; Andreotti et al. 2001; Mougin 2002; Rahman et al. 2002; Germida and Farrell 2003). Organic amendments such as compost may sorb contaminants and improve soil physical and chemical properties, including soil aeration, and may thus increase microbial activity and aid in vegetation establishment (Dick and McCoy 1993; Hill and James 1998; Stratton et al. 1998; Liem et al. 2003).

Many greenhouse and field trials have shown that phytoremediation and bioremediation can successfully reduce petroleum hydrocarbon concentrations in contaminated soils, and the future of these technologies appears promising (Banks et al. 1997; Carman et al. 1998; Hinchman et al. 1998; Frick et al. 1999; National Risk Management Research Laboratory 2000; Andreotti et al. 2001; Stehmeier et al. 2001; Hutchinson et al. 2001a; Mougin 2002; Palmroth et al. 2002; Banks et al. 2003; Bedessem 2003; Siciliano et al. 2003; Singh and Jain 2003). Bioremediation is inexpensive, low-maintenance, simple to implement, well-suited to remote areas and large zones of contamination, compatible with legal requirements to reclaim contaminated sites, socially acceptable and applicable to a wide variety of contaminants; it not only preserves but improves the soil (Lye et al.

1997; Hinchman et al. 1998; Naeth and Howat 1999; Pivetz 2001; Mougin 2002; Palmroth et al. 2002; Banks et al. 2003; McIntyre 2003; Reynolds et al. 2003). The potential of bioremediation for treatment of petroleum hydrocarbon in western Canadian soils thus merits further investigation.

1.2 COMPOSITION AND BIODEGRADABILITY OF PETROLEUM HYDROCARBONS

1.2.1 Composition

Oil products contain contaminants such as asphaltenes, polar compounds and nitrogen-sulphur-oxygen containing organic compounds (NSOs) (Bailey et al. 1973; Song et al. 1990; Salanitro 2001), but are composed mainly of petroleum hydrocarbons. Petroleum hydrocarbons (PHCs) consist of alkanes, alkenes, cycloalkanes, aromatic compounds, heterocycles and polycyclic aromatic hydrocarbons (Atlas 1981; Gustafson et al. 1997; Fathepure and Tiedje 1998; Frick et al. 1999; Salanitro 2001) and are classified into four fractions by the Canadian Council for Ministers of the Environment (CCME) by carbon (C) chain length irrespective of aromaticity (Alberta Environment 2001; ESG International Inc. 2003).

The C_6 to C_{10} fraction comprises approximately 25% of Albertan crude oil by mass (ESG International Inc. 2003). Gasoline, naphtha and natural gas components are included in fraction 1, with significant concentrations of benzene, toluene, ethylbenzene and xylene compounds (BTEX), volatile organic compounds, alkanes and alkylbenzene compounds. The $>C_{10}$ to C_{16} fraction 2 contains kerosene, fuel oil and diesel oil #2 compounds, comprised of alkanes, alkylated polycyclic aromatic hydrocarbons (PAHs), BTEX and alkylated benzenes. Approximately 25% of Albertan crude oil is classified into this fraction. The $>C_{16}$ to C_{34} fraction 3 contains comprises approximately 35% of Albertan crude oil, lubricating oils and greases, heavy fuel oils and road oils. Fraction 3 hydrocarbons include high molecular weight alkanes, naphthalenes, alkylated PAHs, phenanthrenes and fluorenes. The $>C_{34}$ fraction comprises approximately 15% of Albertan crude oil, including asphalts. Fraction 4 is dominated by saturated hydrocarbons, asphaltenes, alkyl chrysenes, steranes and high molecular weight PAHs.

The density of medium gravity crude oil ranges from 0.7 to 0.9 g mL^{-1}, and its composition is approximately 20% alkanes, 4% branched alkanes, 4% cycloalkanes, 4% monoaromatic compounds, 3% PAHs and 0 to 6% heterocyclic compounds. The distribution of saturated, aromatic and heterocyclic fractions in heavy, medium and light crude oils are approximately 20, 29 and 44% in heavy crude oil, 56, 24 and 15% in medium crude oil, and 87, 6 and 0.7% in light crude oil (Salanitro 2001). Westlake et al. (1974) analysed four crude oils from Alaska, Saskatchewan and the Northwest Territories and reported that the aromatic fraction ranged from 30.0 to 39.2% of crude oil composition, compared to 30.5 to 48.5% for saturates.

Alkanes, branched alkanes, low molecular weight cycloalkanes and monoaromatic compounds of C chain length C_4 to C_{15} have high vapour pressures and readily volatilise at ambient temperatures. Since volatilisation is often not measured or accounted for in biodegradation studies, microbial degradation of fraction 1 and 2 compounds is often overestimated (Song et al. 1990; Salanitro 2001; Van Hamme et al. 2003).

Acute and chronic toxicity tests conducted by ESG International Inc. (2003) demonstrated that fraction 2 in Albertan crude oil was the most toxic to plant seeds, seedlings and invertebrates. Toxicity decreased for fraction 3 and whole crude oil, and fraction 4 was the least toxic fraction measured. Alkanes of chain length C_2 to C_{10} are often toxic to microorganisms due to their relatively high solubility in water (Fathepure and Tiedje 1998). The legal standard for total petroleum hydrocarbons (TPH) in Alberta agricultural soils is 1000 mg kg^{-1}, a concentration without adverse effects on soil microorganisms and plants (Environmental Research Advisory Council 2001; Salanitro 2001). Microtox toxicity tests conducted in a field trial in California on soil with weathered heavy oil contamination of 3000 mg kg^{-1} demonstrated no significant change in toxicity with time and vegetation treatments, with effective concentrations (EC_{50}) of approximately 0.3% after 29 months (Banks et al. 2003). The CCME Canada Wide Standards for petroleum hydrocarbons in soil (Canadian Council for Ministers of the Environment 2001) are delineated by fraction and soil texture (Table A1).

1.2.2 Biodegradability of Petroleum Hydrocarbons

Petroleum hydrocarbons are persistent organic pollutants and can remain in contaminated soil for decades (Atlas 1981; Kästner et al. 1994; Salanitro 2001; Van Hamme et al. 2003; White et al. 2003). In field studies in cold climates, Reynolds et al. (2003) demonstrated that the thousands of petroleum hydrocarbons present in contaminated soils degrade at different rates by different enzymes. Atlas (1981) defined weathering as the chemically and biologically induced changes over time in the composition of a polluting petroleum hydrocarbon mixture.

The biodegradability of petroleum hydrocarbons generally decreases in the order: n-alkanes > branched alkanes > branched alkenes > substituted cycloalkanes > monoaromatic hydrocarbons > unsubstituted cycloalkanes > two-ring aromatic hydrocarbons > three-ring aromatic hydrocarbons > four-ring aromatic hydrocarbons > five-ring aromatic hydrocarbons > asphaltenes (Westlake et al. 1974; Atlas 1981; Kästner et al. 1994; Sugiura et al. 1997; Atagana et al. 2003; Chaîneau et al. 2003; Van Hamme et al. 2003). Lighter aromatic hydrocarbons are frequently degraded before heavy saturated compounds (Bailey et al. 1973; Huesemann 1995; Sugiura et al. 1997; Salanitro 2001). Resistance to biodegradation thus increases with structural complexity and molecular mass (Westlake et al. 1978; Atlas 1981; Kästner et al. 1994; Huesemann 1995; Sugiura et al. 1997; Riser-Roberts 1998; Itävaara and Piskonen 2000; Stanley et al. 2000; Salanitro 2001; Atagana et al. 2003; Chaîneau et al. 2003; Van Hamme et al. 2003). Microbial degradation of five-ring aromatic hydrocarbons appears to occur only through co-metabolism (Kästner et al. 1994; Stanley et al. 2000; Salanitro 2001; Van Hamme et al. 2003) by a consortium of microorganisms (Van Hamme et al. 2003; Lors et al. 2004).

Huesemann (1995) examined degradation rates of crude oils of varying compositions in closed landfarming mesocosms over 52 weeks. Potting soils were spiked with three types of crude oil to 5% weight and amended with ammonium nitrate (NH_4NO_3) and potassium hydrogen phosphate (K_2HPO_4) to carbon:nitrogen:phosphorus (C:N:P) ratios of 100:5:1. Initial TPH concentrations for fresh contamination with heavy crude oil and two fresh contamination treatments with lighter crude oils were 2.18, 2.31 and 2.95% dry weight (dw), respectively. Hydrocarbons were analysed as four groups: saturates of carbon numbers <44> and aromatics of carbon numbers <44>. Initial $<C_{44}$ saturate concentrations in heavy crude oil and two lighter crude oil treatments were 0.85, 1.22 and 1.71% dw, respectively, and 66, 86 and 85% of $<C_{44}$ saturates were lost over the experiment. Initial $>C_{44}$ saturate contents were 0.19, 0.29 and 0.35% dw, respectively, with 68, 75 and 76% loss. Aromatic hydrocarbon concentrations in heavy crude oil and two light crude oil treatments were 0.74, 0.46 and 0.56% dw, respectively for $<C_{44}$ aromatics and 0.30, 0.23 and 0.20% dw for $>C_{44}$ aromatics. Respective losses of $<C_{44}$ aromatics were 59, 69 and 72%, and of $>C_{44}$ aromatics were 38, 46 and 44%. Heavier crude oil thus contained higher concentrations of aromatic hydrocarbons and lower concentrations of saturates than lighter crude oils, with little difference in composition or degradation between crude oils of the same gravity (Huesemann 1995). Degradation was lower for aromatic than saturated hydrocarbons and lowest for $>C_{44}$ aromatics.

To be degraded by soil microorganisms and taken up by plants, organic contaminants must come into direct contact with microorganisms and plant roots (Sohrabi et al. 1999; Van Hamme et al. 2003) and thus must be mobile in water. Studies to date indicate that hydrocarbons move into microbial cells passively, without active uptake (Van Hamme et al. 2003). Hydrocarbons with low solubility in water are resistant, though not immune, to mineralisation by soil microorganisms (Kästner et al. 1994; Huesemann 1995; Sugiura et al. 1997; Mougin 2002; Van Hamme et al. 2003). Plant uptake of organic compounds is optimum for compounds of intermediate polarity and moderate hydrophobicity, with log of the octanol-water partition coefficient (log K_{OW}) values of approximately 1.0 to 3.5 (Pivetz 2001; Singh and Jain 2003). Compounds with log K_{OW} values below 1.0 are water-soluble and will move passively into plants through transpiration, but will not phytoaccumulate. Lipophilic compounds of log K_{OW} greater than 3.5 will sorb to plant roots or soil particles; while these compounds will not be absorbed by plants, they may be amendable to microbial degradation in the rhizosphere (Pivetz 2001). Octanol-water partition coefficients of most aromatic petroleum hydrocarbons vary from 2 to 8, while those of aliphatic petroleum hydrocarbons range from 2 to 11 (Gustafson et al. 1997; Salanitro 2001). Phytoaccumulation of petroleum hydrocarbons is thus often insignificant

(Palmroth et al. 2002; Singh and Jain 2003), with mineralisation in the rhizosphere the most significant mechanism of degradation (Frick et al. 1999; Pivetz 2001; Salanitro 2001; Germida and Farrell 2003; Singh and Jain 2003).

Organic xenobiotic chemicals may be sorbed to the surface of humic matter or incorporated into the structure of humic macromolecules (Klavins and Serzane 2000; Salanitro 2001; Mougin 2002; Ke et al. 2003). Formation of these complexes in the aqueous phase can increase solubility and thus mobility of petroleum hydrocarbons. The bioavailability of the contaminant to the microbial community will increase as a result, however. In the solid phase, binding of contaminants to humic material reduces mobility, bioavailability and toxicity of petroleum hydrocarbons (Klavins and Serzane 2000; Salanitro 2001; Mougin 2002; Ke et al. 2003; Van Hamme et al. 2003).

1.3 MICROBIAL ABUNDANCE, DIVERSITY AND ACTIVITY IN HYDROCARBON-CONTAMINATED SOILS

1.3.1 The Soil Microbial Community

A gram of dry soil may contain 10^7 to 10^9 culturable individuals of 40 phenotypes or 4000 genotypes (Trevors 1998). The soil microbial community is diverse and dynamic, and the numbers of a particular species sampled in a soil ecosystem cannot be correlated to its effect upon the soil environment (Wollum 1994; Trevors 1998; Van Hamme et al. 2003). Soils are highly variable, but surface horizons typically support larger and more diverse microbial populations than subsurface horizons (Wollum 1994; Trevors 1998). Soil microbial populations and activities are also spatially variable, correlating with soil physical and chemical properties (Wollum 1994; Trevors 1998; Van Hamme et al. 2003). Microbial community structure and function varies with spatial location, soil depth and time (Wollum 1994; Trevors 1998; Bundy et al. 2002; Sabaté et al. 2004). While most soils are aerobic, anaerobic microsites may form due to low gas diffusion or high rates of oxygen (O_2) consumption (Kaspar and Tiedje 1994; Fathepure and Tiedje 1998).

Many years of research have shown that nutrients, oxygen and water, not petroleum-degrading microorganisms, are limiting in contaminated soils (Westlake et al. 1978; Song and Bartha 1990; Kästner et al. 1994; Huesemann 1995; Riser-Roberts 1998; Sohrabi et al. 1999; Itävaara and Piskonen 2000; Salanitro 2001; Van Hamme et al. 2003; Saul et al. 2005). Many species and genera of soil bacteria and fungi are capable of hydrocarbon utilisation (Atlas 1981; Kästner et al. 1994; Rahman et al. 2002; Van Hamme et al. 2003; Saul et al. 2005), predominantly aerobic (Atlas 1981; Van Hamme et al. 2003). Kästner et al. (1994) enumerated hydrocarbon-degrading bacteria from sites in Germany contaminated with fuel oil or coal tar oil. Microorganisms capable of mineralising PAHs as sole carbon source were found only in soils from contaminated sites, with populations between 10^3 and 10^6 colony forming units (CFU) g^{-1} soil. Huesemann (1995) reported that soils with fresh or weathered crude oil contamination contained 10^9 to 10^{11} CFU g^{-1} total aerobic heterotrophs and 10^6 to 10^9 CFU g^{-1} hydrocarbon degrading microorganisms.

1.3.2 Aerobic and Anaerobic Degradation of Petroleum Hydrocarbons

Anaerobic degradation of hydrocarbons has been reported in many waterlogged or heavily contaminated systems where water or oil saturation limits O_2 diffusion (Atlas 1981; Itävaara and Piskonen 2000; Salanitro 2001; Mougin 2002). The theoretical oxygen demand for aerobic microbial degradation of hydrocarbons is 1 g O_2 for 3.5 g of oil oxidised (Atlas 1981), and oxygen limitation is considered the most important influence on community metabolic function (Song et al. 1990; Lors et al. 2004). Recent studies have shown that aliphatic and aromatic petroleum hydrocarbons can be metabolised in anaerobic conditions by microorganisms using alternate electron acceptors, including sulphate-reducing bacteria, iron-reducing bacteria, denitrifying bacteria and methanogenic archaea (Fathepure and Tiedje 1998; Salanitro 2001; Mougin 2002; Ramsay et al. 2003; Van Hamme et al. 2003), although there is little evidence of anaerobic metabolism of PAHs of four or more rings (Van Hamme et al. 2003). Microbial degradation of toluene and naphthalene under varying redox conditions is described in Table A2.

Hydrocarbon degradation through iron (III) (Fe^{+3}) reduction is limited due to the low bioavailability of Fe^{+3} in soil (Fathepure and Tiedje 1998; Ramsay et al. 2003). Anaerobic metabolism of PHCs by fungi has not been investigated to date (Van Hamme et al. 2003). Ramsay et al. (2003) measured mineralisation of naphthalene and anthracene with various electron acceptors over 160 days. Mineralisation of naphthalene with the electron acceptors O_2, nitrate (NO_3^-), Fe^{+3} and sulphate (SO_4^{-2}) was approximately 35, 30, 12 and 30%, respectively. Mineralisation of anthracene was slower, with totals of 8.5, 5.8, 3.7 and 4.9% mineralisation in the presence of electron acceptors O_2, NO_3^-, Fe^{+3} and SO_4^{-2}, respectively. No methane (CH_4) production was detected in the aerobic or anaerobic incubations. The iron-reducing bacteria utilised insoluble FeOOH as a terminal electron acceptor as efficiently as soluble Fe^{+3} (Ramsay et al. 2003). Manganese (Mn^{+2}) and humic acids may also serve as terminal electron acceptors for anaerobic PHC degradation (Van Hamme et al. 2003).

Aerobic and anaerobic biodegradation of historical mineral oil contamination was studied in soil samples from a site with shallow groundwater or perched groundwater tables of 0.8 to 2.5 m (Salminen et al. 2004). Soil samples from 0.55 to 2.55 m depth, with mineral oil concentrations of 750 to 7800 mg kg^{-1}, were incubated under aerobic and anaerobic conditions for 4 and 10 months, respectively. No anaerobic activity was detected to 0.55 m depth, but removal of mineral oil with anaerobic degradation at 1.05 to 2.55 m ranged from 6 to 38%. Approximately 0.2% of this oil was converted to CH_4, 2 to 33% was mineralized as carbon dioxide (CO_2), and 20 to 99% was incorporated as organic matter. Aerobic degradation yielded 15 to 44% removal of mineral oil, of which 10 to 60% was mineralised as CO_2 and 20 to 99% was converted to organic matter. Aerobic activity was low at this site, and CO_2 production during anaerobic incubation suggests that Fe^{+3} or SO_4^{-2} reduction were the dominant anaerobic processes.

Soil samples from a second, more heavily contaminated sampling location on the same site were taken at 1.75 to 3.4 m depth and yielded mineral oil concentrations of 5000 to 16 400 mg kg^{-1}, increasing with depth (Salminen et al. 2004). Anaerobic degradation removed 34 to 63% of mineral oil, of which 27 to 59% was converted to organic matter, 4 to 70% as CH_4 and 3 to 20% as CO_2. Under aerobic incubation, mineral oil concentrations were reduced by 31 to 44%. Of the mineral oil removed, 39 to 97% was incorporated into organic matter and 16 to 28% was mineralised as CO_2. Methanogenesis was the dominant anaerobic process at this location; however, high aerobic and anaerobic activity was measured in the same samples.

1.3.3 Hydrocarbon Degrading Bacteria In Soils

Proportions of hydrocarbon-degrading microorganisms in soil communities increase with hydrocarbon exposure, constituting less than 0.1% in unpolluted ecosystems to up to 100% in oil-contaminated ecosystems (Walker and Colwell 1976; Atlas 1981; Song and Bartha 1990; Kästner et al. 1994; Lindstrom et al. 1999; Rahman et al. 2002; Chaîneau et al. 2003; Sabaté et al. 2004; Saul et al. 2005). Semenov et al. (1998) determined that 50 mL crude oil kg^{-1} was the threshold concentration for inducing domination of hydrocarbon-oxidising microorganisms in freshly contaminated soil. Total bacterial populations in Alaskan boreal forest did not differ significantly from those in reference soils almost 20 years after a crude oil spill, but numbers of actively respiring bacteria were significantly lower (Lindstrom et al. 1999). In contrast, hydrocarbon-degrading bacteria comprised 1 to 3% of total bacteria in contaminated soils but at most 0.005% in reference non-contaminated soils. Substrate utilisation patterns suggested weathered crude oil contamination reduced microbial population diversity, but microorganisms in this contaminated soil utilised a wider range of substrates (Lindstrom et al. 1999).

Sabaté et al. (2004) reported that 19.3% of heterotrophic microorganisms in weathered petroleum-contaminated soil were hydrocarbon degraders. Hydrocarbon degrading microorganisms were estimated to comprise 0.01% of the total aerobic heterotrophic population in non-contaminated loam soil sampled by Song and Bartha (1990), but this value increased to 90.8 and 70.6% two weeks after application of 50 and 135 mg g^{-1} jet fuel, respectively. These values had dropped to 18.8 and 9.4% by the end of the 16-week experiment, corresponding with trends in the total microbial population (Song and Bartha 1990). Contamination with jet fuel caused an initial increase in the aerobic heterotrophic populations, but after 16 weeks they had dropped to slightly below those at the start of the trial. Hydrocarbon degrading microorganisms, however, remained more than two orders of magnitude above initial values. Liming, fertilisation and tilling increased total aerobic heterotrophic and hydrocarbon degrading populations in contaminated soil by one and three orders of magnitude, respectively (Song and Bartha 1990).

In Antarctic soil with weathered contamination from hydraulic and lubricating oils, Saul et al. (2005) counted 1.4 x 10^7 CFU g^{-1} culturable aerobic heterotrophs, 1.3 x 10^6 CFU g^{-1} dodecane degrading microorganisms and 4.1 x 10^6 CFU g^{-1} phenanthrene degrading microorganisms. Adjacent non-contaminated soils contained 4.1 x 10^5 CFU g^{-1} culturable aerobic heterotrophs and <10 CFU g^{-1} hydrocarbon degraders, indicating hydrocarbon-degrading microorganisms dominated in contaminated soil. Enumeration of total soil microorganisms using epifluorescence microscopy yielded 10.0 and 4.9 x 10^7 total counts g^{-1} in control and contaminated soils, respectively.

1.3.4 Hydrocarbon Degradation by Fungi

Mixed microbial populations of bacteria, yeasts and fungi are required for thorough degradation of petroleum hydrocarbons, and in most soils the indigenous microbial community contains sufficient population density and diversity for successful petroleum bioremediation (Westlake et al. 1978; Kästner et al. 1994; Huesemann 1995; Lye et al. 1997; Sugiura et al. 1997; Riser-Roberts 1998; Sohrabi et al. 1999; Itävaara and Piskonen 2000; Salanitro 2001; Rahman et al. 2002; Van Hamme et al. 2003; Sabaté et al. 2004). While the abilities of bacteria and yeast to degrade hydrocarbons appear to decrease with increasing carbon chain length, fungal degradation of hydrocarbons does not appear to be as influenced by carbon chain length (Atlas 1981). Fungal metabolism of petroleum hydrocarbons is of great interest because fungi can produce extracellular enzymes and can, through fungal hyphae, access hydrocarbons in hydrophobic soils and soil aggregates that would be unavailable to bacteria (April et al. 2000).

Studies of white rot fungi (a physiological grouping) have shown promise (Semenov et al. 1998; Stanley et al. 2000). Song and Bartha (1990) reported that application of jet fuel to soil columns increased lengths of fungal hyphae from 1 x 10^7 to 5 x 10^9 m g^{-1} soil after an initial small decrease; no effect was observed at the bottom of soil columns. A combined liming, fertilisation and tilling treatment increased lengths to 10^{10} m g^{-1}. Hydrocarbon degrading fungi isolated from Canadian flare pits demonstrated aliphatic PHC degradation, but none were able to degrade aromatic PHCs (April et al. 2000). Fungi capable of degrading crude oil alkanes included species from the genera *Aspergillus*, *Beauveria*, *Fusarium*, *Neosartorya*, *Oidiodendron*, *Paecilomyces*, *Phialophora* and *Pseudallescheria*, with species of *Penicillium* exhibiting the greatest ability to degrade aliphatic hydrocarbons. The ability of these fungi to mineralise PHCs was analysed by radiorespirometry with radiolabeled hexadecane and phenanthrene. Recovery of hexadecane ^{14}C as $^{14}CO_2$ ranged from 0.5 to 37.1%, while 1.4 to 11.6% was recovered as biomass; these results were attributed in part to adsorption to mycelium. No mineralisation of radiolabeled phenanthrene was observed. Results suggested that fungi may partially break down PHCs to smaller or more soluble compounds for degradation by other microorganisms (April et al. 2000).

1.3.5 Metabolic Pathways for PHC Biodegradation

Metabolic pathways vary between compound and microorganisms, and are not well understood for fungi and many bacterial species (Itävaara and Piskonen 2000; Van Hamme et al. 2003). Soil microorganisms may utilise hydrocarbons for energy or carbon, metabolising the contaminants into CO_2, or may co-metabolise the contaminants during degradation of other organic compounds (Nichols et al. 1997; Sugiura et al. 1997; Semenov et al. 1998; Salanitro 2001; Mougin 2002; Van Hamme et al. 2003). Alkanes and aromatic compounds of low molecular weight can be metabolised directly to CO_2 and microbial biomass, but PAHs are often only partially degraded. Soil microorganisms may detoxify large and structurally complex contaminants into smaller, stable compounds, and these partially oxidised products will accumulate in weathered soils (Atlas 1981; Itävaara and Piskonen 2000; Salanitro 2001; Mougin 2002; Chaîneau et al. 2003; Van Hamme et al. 2003; Sabaté et al. 2004). Intermediate compounds such as waxes and tar balls may be produced during microbial degradation of petroleum hydrocarbons (Atlas 1981; Van Hamme et al. 2003).

Many hydrocarbons that would not be metabolised in isolation will degrade in mixtures of petroleum hydrocarbons, an effect attributed to co-oxidation (Atlas 1981; Itävaara and Piskonen 2000; Salanitro 2001; Rahman et al. 2002; Van Hamme et al. 2003; Lors et al. 2004). Enzymes from more than one species of microorganisms may be required to complete the metabolic pathway of a petroleum hydrocarbon, requiring a diverse population of soil microorganisms. Toxic soil contamination often reduces species richness and thus diversity, but domination of microorganisms capable of metabolising the contaminant will also reduce soil biodiversity (Trevors 1998; Saul et al. 2005). Information on the effect of PHCs on microbial population diversity is incomplete, however (Trevors 1998).

1.3.6 Research To Date

Ninety days after adding 100 mg kg^{-1} PAHs to silty clay loam soil, Maliszewska-Kordybach and Smreczak (2003) observed a slight but significant increase in the total bacterial population from 479 to 508 x 10^6 CFU g^{-1}, a slight but significant drop in dehydrogenase activity from 360 to 264 mm^3 H_2 100 g^{-1}, and no significant differences in acidic phosphatase activity or CO_2 respiration rate between treatments. Rahman et al. (2002) reported that bacterial populations in soils contaminated with diesel fuel were dominated by *Corynebacterium* (36.5% of culturable population), *Pseudomonas* (14.4%), *Micrococcus* and *Bacillus* (11.1% each), with smaller (<10%) concentrations of *Enterobacter*, *Flavobacterium*, *Moraxella*, *Alcaligenes*, *Aeromonas*, *Acinetobacter* and *Vibrio*. These communities consisted of 51.1% catalase reducers and of 68.2% ammonia producers, 60.3% nitrite reducers and 44.3% nitrate reducers, all three of which contribute to nitrogen (N) cycling (Myrold 1999). Nitrite and nitrate reducers were somewhat more dominant in gasoline-contaminated soils (Rahman et al. 2002).

Microbial populations in non-contaminated soil and soil from a former coke site were compared by Lors et al. (2004). The contaminated soil, with 3931 mg kg^{-1} PAH and elevated heavy metal concentrations, had much higher total organic N and total phosphorus (P) content than the reference soil; no explanation was offered. Bacterial populations in contaminated and reference soils were 1.8 and 0.3 x 10^6 CFU g^{-1}, respectively, and respective fungal populations were 74 and 1.5 x 10^3 CFU g^{-1}. Both soils contained approximately 10^5 CFU g^{-1} naphthalene degrading microorganisms, but only contaminated soil held microorganisms capable of degrading anthracene, fluorine, phenanthrene, fluoranthene or pyrene. Eight percent of bacteria in PAH-contaminated soil were Gram-positive, compared to 53% in non-contaminated soil; the dominance of Gram-negative bacteria was attributed to the physical protection against contaminants provided by the outer membrane (Lors et al. 2004), which may limit the ability of Gram-negative bacteria to access hydrocarbon substrates (Sugiura et al. 1997). PAH-degrading bacteria isolated from contaminated soil belonged to the genera *Ochrobacter*, *Pseudomonas*, *Flavomonas*, *Aeromonas*, *Comamonas*, *Moraxella*, *Photobacterium* and two unidentified Gram-negative species. Four naphthalene-degrading species were isolated from the reference soil: *Agrobacterium*, *Corynebacterium*, *Chryseobacterium* and an unidentified Gram-positive species (Lors et al. 2004).

Nichols et al. (1997) studied rhizosphere microbial populations in petroleum contaminated sandy loam soil from Alaska. This soil was contaminated to 2000 mg kg^{-1} with a mixture of hexadecane, 2,2-dimethylpropyl benzene, *cis*-decahydronaphthalene, benzoic acid, phenanthrene and pyrene and incubated for 14 weeks. The native grass *Poa alpina* L. (alpine bluegrass) was grown in selected soil treatments. Rhizosphere soil was sampled for hydrocarbon-degrading microorganisms using the most probable number (MPN) method, while bacterial and fungal populations were measured using plate counting. Populations of hydrocarbon-degrading microorganisms in non-vegetated treatments were significantly higher in contaminated than non-contaminated soils after 14 weeks, with means of 6.39 and 5.46 log MPN g^{-1} soil, respectively. At nine weeks, hydrocarbon-degrading microorganisms in the rhizosphere of *P. alpina* were 7.00 log MPN g^{-1} contaminated soil; populations in bulk soil of vegetated, contaminated treatments and in rhizosphere soil of non-contaminated treatments were slightly lower at 6.70 and 6.06 log MPN g^{-1} soil, respectively. Bacterial populations in contaminated, non-vegetated soil increased significantly over 4 weeks from 7.34 to 7.92 log CFU g^{-1}, but the change was not significant after 14 weeks. Fungal populations dropped from 4.73 to 4.49 log CFU g^{-1} over 14 weeks, due possibly to competition with bacteria. Both populations declined in non-contaminated, non-vegetated soil, and results from both populations were considered to be consistent with results from other studies (Nichols et al. 1997).

Establishment of *Poa alpina* in contaminated soil did not significantly affect bacteria or fungi populations in the rhizosphere as compared to bulk soil (Nichols et al. 1997). Percentages of bacterial populations that were hydrocarbon degraders in vegetated, contaminated soil were 21.9 and 12.4% in the rhizosphere and bulk soil, respectively, compared to 13.7 and 5.2%, respectively, in vegetated, non-contaminated soils (Nichols et al. 1997). These results demonstrate a significant shift in the microbial community upon vegetation establishment, suggesting biodegradation of hydrocarbon contaminants was stimulated by plant roots.

Bailey and McGill (2001) studied respiration in two soils with weathered petroleum contamination: Devon loam soil with 18% dichloromethane extractable organic carbon (DEO-C) and low electrical conductivity (EC), and Montreal clay loam soil with 8% DEO-C and 5 dS m^{-1} EC. Three rates of nutrient addition were tested, based upon the amount of N, P and sulphur (S) required for degradation of organic material. The cumulative carbon respired from the Devon and Montreal soils after 10 weeks incubation without aeration at the normal (theoretical requirement) nutrient amendment rate was 1.97 and 0.17 μg CO_2-C $(g\ soil)^{-1}$, respectively. Carbon dioxide evolution was best represented by a first-order exponential model, in which substrate availability was the only limiting factor. In contrast, Lindstrom et al. (1999) reported that soil respiration rates in Alaskan soil contaminated over 20 years earlier with crude oil did not differ significantly from those in non-contaminated soil.

Populations of heterotrophic bacteria and hydrocarbon-adapted bacteria were monitored in windrows of freshly contaminated soil over two years by Chaîneau et al. (2003), using MPN methods. Addition of crude oil initially increased microbial populations, as did fertiliser and straw amendment to contaminated windrows. After 180 days, numbers of heterotrophic bacteria in non-amended windrows, fertilised windrows and windrows amended with fertiliser and straw were 9.50, 200 and 4500 x 10^6 CFU g^{-1} soil, respectively. Counts of hydrocarbon-adapted bacteria in these respective treatments were

9.50, 45 and 75 x 10^6 CFU g^{-1}. Heterotrophic and hydrocarbon-adapted bacterial populations in non-contaminated soil were 2.5 x 10^6 and 0.025 x 10^6, CFU g^{-1}, respectively (Chaîneau et al. 2003), indicating oil contamination increased the proportion of hydrocarbon-adapted bacteria in the soil from 0.1 to 100%. The lowered relative abundance of hydrocarbon-adapted bacteria in windrows amended with fertiliser and with fertiliser and straw, calculated at 22 and 1.7%, respectively, after 180 days, was not discussed. Microbial populations were correlated with decreases in toxicity and crude oil contamination (Chaîneau et al. 2003), however, suggesting the relative abundance of hydrocarbon-degrading organisms decreased as the overall microbial population increased. The term "hydrocarbon-adapted bacteria" was not defined.

Podzolic sandy loam soil and fluvisolic loamy sand were incubated for 103 days with no contamination or fresh 1% dw diesel fuel (Bundy et al. 2002). N and P were added to adjust the oil:N:P ratio to 100:10:1, approximately 100:12:1.25 C:N:P. Diesel fuel loss was 60%, corresponding to increased aerobic respiration rates and microbial biomass. Respiration peaked for the sandy loam soil by day 27 and had decreased to near-control levels by the end of incubation, while the loamy sand did not reach maximum respiration until day 62 and did not decline to control levels. Microbial biomass also reached its maximum value by day 27 for contaminated sandy loam soil, and declined but remained elevated over those in control soils. Loamy sand had lower microbial biomass than the sandy loam soil in both contamination treatments. Diesel fuel also increased microbial biomass for loamy sand, with the maximum value measured at the end of the experiment. Phospholipid fatty acid profiling (PLFA) indicated that the microbial communities in the soils were distinct from each other, as were contaminated soils from control treatments. Community profiles for contaminated and control treatments remained distinct at the end of the experiment, with contaminated samples diverging from each other throughout the trial; diesel fuel contamination was the main source of variation between samples, and served to differentiate communities between the soil types (Bundy et al. 2002).

1.3.7 Conditions and Kinetics of PHC Metabolism

Microbial metabolism is highly dependant upon temperature, but hydrocarbon biodegradation has been observed at temperatures below 0 °C (Atlas 1981; Itävaara and Piskonen 2000; Salanitro 2001; Seklemova et al. 2001; Rahman et al. 2002). Chaîneau et al. (2003) showed that bioremediation of fresh crude oil in windrowed soil was possible at temperatures of 10 °C. Enzymes produces by cold-adapted microorganisms have more flexible protein structures and higher activities at lower temperatures compared to mesophilic microorganisms (Itävaara and Piskonen 2000). Optimum conditions for biodegradation vary with sites and microbial populations. Chang et al. (2001) reported that optimal conditions for biodegradation of phenanthrene were 30 °C, pH 7.0 and soil water content of 100% weight basis (w/w), while Atagana et al. (2003) found pH 6.5 to 7.0 and soil water content of 70% w/w were optimal for biodegradation of weathered creosote contamination. Sohrabi et al. (1999) recommended soil moisture levels of 25 to 85% of field capacity. Hydrocarbon-degrading bacteria isolated from soils at gasoline and diesel fuelling stations had higher growth in culture at pH 7.5 than at pHs of 6.5 or 8.5 (Rahman et al. 2002). Successful bioremediation of hydrocarbon contaminants in soil have been reported at sites across Canada (Lye et al. 1997).

Microbial degradation of petroleum hydrocarbons follows first-order kinetics, in which amount of substrate consumed is proportional with amount of time passed; degradation rates, rate constants and half-lives are documented in the literature (Huesemann 1995; Riser-Roberts 1998; Song et al. 1990; Sohrabi et al. 1999; Bailey and McGill 2001; Brook et al. 2001; Chang et al. 2001; Salanitro 2001; Namkoong et al. 2002). Experiments demonstrated that aerobic biodegradation of petroleum hydrocarbons at high hydrocarbon concentrations follow Monod growth (Sohrabi et al. 1999) and Michaelis-Menton reaction rate kinetics (Riser-Roberts 1998), in which growth and reaction velocity are dependent upon substrate concentration. Kinetics of biodegradation at low substrate concentrations are less well understood (Riser-Roberts 1998), although Song et al. (1990) described moderate increases in half-lives with higher fuel concentrations.

1.4 PHYTOREMEDIATION OF PETROLEUM HYDROCARBONS: THE RHIZOSPHERE EFFECT

A literature review by Frick et al. (1999) found that vegetation stimulates microbial mineralisation of petroleum hydrocarbons primarily through the rhizosphere effect. This term describes the increased microbial populations and activity in the rhizosphere from carbohydrate, amino acid, carboxylic acid, enzyme, chelating agent, nutrient and some oxygen exudation from plant root systems (Aprill and Sims 1990; Banks et al. 1997; Kosimar and Park 1997; Nichols et al. 1997; Hinchman et al. 1998; Trevors 1998; Adam and Duncan 1999; Frick et al. 1999; Alkorta and Garbisu 2001; Pivetz 2001; Davis et al. 2002; Mougin 2002, Banks et al. 2003; Singh and Jain 2003; Van Hamme et al. 2003; White et al. 2003). This supply of root exudates to bacteria and fungi in the rhizosphere stimulates growth and metabolism of soil microbial communities, resulting in higher rates of hydrocarbon degradation in vegetated soils than in non-vegetated soils (Aprill and Sims 1990; Lee and Banks 1993; Banks et al. 1997; Kosimar and Park 1997; Nichols et al. 1997; Nedunuri et al. 2000; Alkorta and Garbisu 2001; Hou et al. 2001; Hutchinson et al. 2001b; Pivetz 2001; Davis et al. 2002; Palmroth et al. 2002; Banks et al. 2003; Ke et al. 2003; Reynolds et al. 2003; Siciliano et al. 2003; Singh and Jain 2003; Van Hamme et al. 2003). Leigh et al. (2002) found that 58% of the fine roots produced by *Morus* L. (mulberry) in a six-month growing season died at the end of the season, serving as a potential source of substrate for bacteria which consume recalcitrant organic pollutants and promoting further degradation of soil contaminants.

Improvement in soil physical and chemical properties through vegetation establishment, including reduction of infiltration and erosion, soil stabilisation and improved soil aeration, also improve rates of bioremediation (Riser-Roberts 1998; Frick et al. 1999; Hutchinson et al. 2001b; Pivetz 2001; Davis et al. 2002; Mougin 2002; Banks et al. 2003; Reynolds et al. 2003). The latter is of particular importance in wetland systems and saturated soils, because O_2 is often the limiting agent in in situ bioremediation; improved soil aeration through improved soil structure and oxygen delivery to the rhizosphere prevents anaerobic conditions from developing in the soil (Lye et al. 1997; McIntyre 2002). Root penetration can break up soil aggregates and small pores, exposing trapped contaminants to soil microorganisms, and increase water and air infiltration into contaminated soils (Banks et al. 2003). Hutchinson et al. (2001b) reported that aged petroleum sludge vegetated with *Festuca arundinacea* Schreb. (tall fescue) exhibited soil structure, aggregation and improved drainage in comparison to non-vegetated sludge. These strong effects yielded greater O_2 diffusion and water infiltration over seven months in this greenhouse experiment (Hutchinson et al. 2001b).

Plants may release enzymes into the rhizosphere which degrade organic chemicals directly (Frick et al. 1999; Alkorta and Garbisu 2001; Davis et al. 2002; Banks et al. 2003; Van Hamme et al. 2003). In turn, soil microorganisms break down toxic chemicals to reduce phytotoxicity to the plants growing in contaminated soil (Frick et al. 1999; Andreotti et al. 2001). Some plants accumulate or metabolise organic contaminants, while others take up organic compounds from the soil solution and release them into the air through evapotranspiration. Plants such as *Medicago sativa* L. (alfalfa), *Salix* L. (willow) or *Populus* L. (poplar) which transpire high amounts of water are highly efficient in this phytovolatilisation process, and may be used to prevent migration of contaminants from the affected area (Hinchman et al. 1998; Frick et al. 1999; National Risk Management Research Laboratory 2000; Davis et al. 2002). Plant uptake of petroleum hydrocarbons is often negligible (Frick et al. 1999; Van Hamme et al. 2003) and while many plants hyperaccumulate metals, none have been discovered which hyperaccumulate PHCs (Singh and Jain 2003).

1.5 NUTRIENT REQUIREMENTS AND ADDITION IN BIOREMEDIATION

1.5.1 Nitrogen and Phosphorus Demand in Hydrocarbon-Contaminated Soils

The effects of inorganic nutrient application on hydrocarbon degradation have been well studied (Riser-Roberts 1998; Salanitro 2001; McIntyre 2002; Chaîneau et al. 2003; Van Hamme et al. 2003). Nitrogen and P are often the dominant limiting factors in biodegradation of hydrocarbons in soil (Westlake et al. 1978; Riser-Roberts 1998; Sohrabi et al. 1999; Van Hamme et al. 2003). Addition of inorganic fertiliser increases rates of hydrocarbon degradation by supplying N and P to the soil, restoring the nutrient balance of these nutrients with C (Westlake et al. 1978; Atlas 1981; Song et al. 1990; Margesin and Schinner 1997; Riser-Roberts 1998; Ka et al. 2001; Margesin and Schinner 2001; Pichtel and Liskanen 2001; Salanitro 2001; McIntyre 2002; Chaîneau et al. 2003).

Plate counts of bacteria from boreal soil at Norman Wells, Northwest Territories, which had been freshly contaminated with crude oil, showed that application of 600 kg N ha^{-1} significantly increased viable bacteria populations (Westlake et al. 1978). A hundredfold increase was observed after 22 days; this difference slowly decreased with time, but bacterial populations were still significantly higher in fertilised treatments after three years (Westlake et al. 1978). These results were matched by decreases in the saturate fraction of crude oil contamination, little decrease in asphaltenes, no decrease in aromatic content and an increase in NSO compounds. Liming, tilling and application of N and P fertilisers reduced net average doubling rates of hydrocarbon-degrading microorganisms from 30 to 20 hours in loam soil contaminated with jet fuel (Song and Bartha 1990). Hutchinson et al. (2001a) found degradation of aged petroleum sludge occurred at the highest rates when N and P were supplied to soil at rates in excess of amounts required to maintain plant growth. The optimal nutrient balance for petroleum hydrocarbon degradation in this short-term, greenhouse study was a C:N:P ratio of 500:10:1. Ka et al. (2001) reported addition of N and P increased microbial metabolic activity (measured by rRNA concentration), but not population density, in arctic soil contaminated with fuel.

Degradation of petroleum hydrocarbons by soil microorganisms proceeds at relatively slow rates in cold northern climates (Atlas 1981; Salanitro 2001), and Margesin and Schinner (2001) found that while natural attenuation of diesel fuel contamination in an alpine glacial area reduced hydrocarbons by 50% over three years, residual concentration of hydrocarbons remained high at 1296 mg kg^{-1}. Low rates of inorganic fertiliser application by Palmroth et al. (2002) failed to significantly increase degradation of diesel fuel. Bioremediation of historical diesel oil contamination at a site in northern Alberta demonstrated a 46% decrease in total extractable hydrocarbons two years after application of 0.336 Mg ha^{-1} of 20-3-4 NPK (nitrogen-phosphorus-potassium) fertiliser, compared to an 8% decrease in the unfertilised control (McIntyre 2002). Saul et al. (2005) compared total N and P concentrations between control and oil-contaminated soils from Antarctica and found that while total N concentrations were very low at <0.01%, total P concentrations were high at 0.19%.

1.5.2 Nutrient Ratios

The ratio of C to N in soil undergoing bioremediation may be the most important factor in nutrient stimulation of biodegradation (Riser-Roberts 1998; Salanitro 2001). Rosenberg et al. (1992) calculated that 150 mg N and 30 mg P is required to convert 1 g of hydrocarbon to cellular material. Recommended C:N ratios vary from 1.7:1 to 200:1, and recommended C:P ratios from 17:1 to 1000:1 (McIntyre 2002). Salanitro (2001) suggested nutrient addition may fail to increase PHC degradation due to direct mineralisation without cell growth, limited bioavailability of PHCs in soil and incomplete breakdown of PHCs. Optimal results have been reported in bioremediation trials on petroleum-contaminated sites at a C:N ratio of 60:1 and a C:P ratio of 800:1 (Riser-Roberts 1998), yielding a C:N:P ratio of 800:13:1. Sohrabi et al. (1999) recommended a C:N:P ratio of 120:10:1. Beaudin et al. (1999) reported total degradation of mineral oil and grease in weathered hydrocarbon-contaminated soil composted with plant material increased significantly as C:N ratios decreased from 49 to 17. Diesel fuel degradation was dependent upon N source and C:N ratio (Brook et al. 2001), with higher TPH degradation rates at C:N 40:1 using NO_3^- fertilisers and at C:N 20:1 using urea and ammonium sulphate ($(NH_4)_2SO_4$) fertiliser. Phosphorus concentrations were adjusted to 200:1 for each treatment.

Soil with high, weathered creosote contamination and C:N:P ratio of 130:0.08:4.5 was amended to C:N ratios of 25:1, 20:1, 15:1, 10:1 and 5:1 in a soil microcosm experiment (Atagana et al. 2003). Creosote removal from these respective treatments after six weeks was 68.7, 61.1, 56.3, 63.8, 50.2 and 33%, compared to 46.6% in a non-amended control and 18.2% in a sterilised control. These values were positively correlated with increasing populations of hydrocarbon-degrading microorganisms, measured by plate counting. The highest C:N ratio tested, 25:1, was optimal under these conditions. Phosphorus addition of C:P 10:2 did not significantly improve creosote degradation in the C:N 10:1 treatment.

In field trials at sites with historical petroleum contamination, the Remediation Technologies Development Forum (RTDF) Phytoremediation Action Team applied N and P fertiliser to vegetated plots at a C:N ratio of 50:1 and a N:P ratio of 100:1 (Kulakow 2000; Kulakow and Erickson 2001), yielding a C:N:P ratio of 5000:100:1. The field study protocol developed by the RTDF Phytoremediation Action Team calls for fertilisation at a C:N:P ratio of 50:2:1 (RTDF 1999).

1.6 ORGANIC AMENDMENTS IN BIOREMEDIATION OF HYDROCARBONS

1.6.1 Research To Date

Liem et al. (2003) reported that application of humic substances to soil freshly contaminated with diesel significantly improved moisture retention, particle size distribution, pH, C:N ratio, growth of heterotropic bacteria and hydrocarbon biodegradation. At a current phytoremediation project being conducted on excavated flare pit soil in Saskatchewan, straw, manure and gypsum were applied to the sodic (sodium adsorption ratio of 25) clay soil to improve soil structure and water infiltration (Germida and Farrell 2003). Composted manure was then spread and packed on the site as a seedbed. Early results indicated that CCME fraction 2, 3 and 4 hydrocarbons were dropping over non-amended control treatments in non-seeded plots, but were unclear for seeded plots (Farrell and Germida 2004). Final results have not been published to date.

Biopiles of sandy soil with weathered oily sludge contamination from a waste site near Montreal, Québec, were amended with activated sludge to 0.2% weight basis (Juteau et al. 2003). The biopiles had reductions in concentrations of fraction 3 PHCs chrysene and benzo(a)anthracene, while concentrations in non-amended soils did not significantly change. Chrysene concentrations decreased from 12.6 to 1.8 mg kg^{-1} after 126 days, while those of benzo(a)anthracene decreased from 12.6 to 1.2 mg kg^{-1}. Fraction 2 PHCs 1-methyl-naphthalene, 2-methyl-naphthalene and phenanthrene were fully removed in both amended and non-amended treatments, indicating sludge amendment improved microbial degradation specifically of fraction 3 hydrocarbons.

Many years of research have shown that addition of compost to disturbed soil can improve soil physical, chemical and microbiological properties (Dick and McCoy 1993; Hill and James 1998; Stratton et al. 1998). Compost application adds organic matter to soil and thus stimulates soil microbial populations, which in turn improves aggregate stability, soil water retention, porosity and aeration. The nutrients in compost can reduce plant requirements for inorganic fertilisers by one-third to one-half (Dalzell et al. 1987; Sikora and Enkiri 1999). Compost acts as a bulking agent to improve aeration, thus stimulating biodegradation of hydrocarbons by improving O_2 availability to soil microorganisms (Brown et al. 1998; Vasudevan and Rajaram 2001). The organic matter in compost may also sorb petroleum hydrocarbons, reducing their bioavailability (Kästner and Mahro 1996; Verstraete and Devliegher 1996; Vouillamoz and Milke 2001). Humic acids in compost may act as surfactants for soil microorganisms, increasing absorption of nutrients and substrates (Vallini et al. 1997). Compost may also provide the co-substrates required for co-metabolism of PHCs (Van Hamme et al. 2003).

Microbial degradation of hydrocarbon contaminants and plant growth in contaminated soil can thus benefit from application of compost (Kästner et al. 1995; Kästner and Mahro 1996; Brown et al. 1998; Chang et al. 2001; Vasudevan and Rajaram 2001), but little field work has been done to evaluate the potential of compost as an organic amendment in phytoremediation of PHCs. Of the reviewed studies conducted with compost, analysis was limited to total hydrocarbon content (Hupe et al. 1996; Lye et al. 1997; Vasudevan and Rajaram 2001; Vouillamoz and Milke 2001; Namkoong et al. 2002) or to CCME fraction 1 and 2 PHCs (Chang et al. 2001; Kästner et al. 1995; Kästner and Mahro 1996). Most studies were limited to controlled laboratory conditions, the results of which may not be directly applicable to field conditions (Davis et al. 2003).

Hupe et al. (1996) demonstrated that compost application to diesel-contaminated soil increased O_2 consumption by hydrocarbon-degrading microorganisms and resulted in 94% reduction of diesel fuel contaminants. In a 162-day trial with lubricating oil contamination, more than 75% of contaminants disappeared from soil amended with compost compared to 37% in non-amended soil. Various treatments were tested on excavated hydrocarbon-contaminated soil from a fire-fighter training area at Canadian

Forces Base Trenton, in southern Ontario, from July to November 1995 (Lye et al. 1997). Combinations of compost with inorganic fertiliser, poultry manure or white rot fungi, with or without turning soil piles, reduced TPH concentrations to acceptable levels by most provincial criteria. Further information was not provided.

Brown et al. (1998) conducted bench-scale experiments with weathered, hydrocarbon-contaminated soil from an inactive oil refinery. Methylated alkanes (pristane and phytane) disappeared rapidly over 110 days from soil amended with high N sewage sludge compost; degradation rates were lower in soils amended with sawdust or sludge compost of low N concentration. Nearly half the complex branched and cyclic alkanes in soil treated with high N compost disappeared as well. Depletion of dibenzothiophenes and phenanthrenes was 75 and 90%, respectively, after 70 days.

Namkoong et al. (2002) combined mature food waste compost at varying ratios with soil freshly contaminated to 10 000 mg kg^{-1} diesel fuel. Ratios of contaminated soil to compost on a wet weight basis were 1:0.1, 1:0.3, 1:0.5 and 1:1. The mixtures were composted in a compost bioreactor with aeration 200 L min^{-1} m^{-3} soil, and volatilisation and evolved CO_2 monitored. Volatilisation measured 0.9 to 2.6% across treatments while hydrocarbon degradation in biocide treated mixtures was negligible, indicating that disappearance of PHCs from contaminated soil was due to biodegradation. Petroleum hydrocarbon degradation was greatest at a mixture ratio of 1:0.5 of soil:compost, with 99.6% removal over the 30 day trial period. Non-amended, contaminated soil had 66.6% removal of TPH over the same period. The first-order reaction kinetic constant k at this ratio was 0.104 for TPH, compared to 0.037 for non-amended soil (Namkoong et al. 2002). Correlation coefficients for cumulative CO_2 evolved and dehydrogenase activity to TPH concentration degradation were 0.86 and 0.82, respectively, indicating that PHC loss was caused by mineralisation by soil microorganisms.

Amendment of loamy sand with compost at 1:1 weight ratio increased total organic carbon (TOC) from 0.75 to 5.20%, total bacterial population from 20 to 142 x 10^6 CFU g^{-1}, dehydrogenase activity from 305 to 2661 mm^3 H_2 100 g^{-1}, acidic phosphatase activity from 38 to 168 µg *p*-nitrophenyl g^{-1}, and intensity of respiration from 51 to 295 µg C-CO_2

g^{-1} (Maliszewska-Kordybach and Smreczak 2003). These increases remained after 60 days incubation with 10 mg kg^{-1} three- and four-ring PAH contamination, raising total bacterial population from 101 to 370 x 10^6 CFU g^{-1}, dehydrogenase activity from 448 to 2588 mm^3 H_2 100 g^{-1}, acidic phosphatase activity from 20 to 126 µg *p*-nitrophenyl g^{-1}, and intensity of respiration from 60 to 300 µg C-CO_2 g^{-1}. Contamination increased total bacteria and slightly decreased dehydrogenase and acidic phosphatase activity in both soils, but increased CO_2 respiration in the compost treatment only (Maliszewska-Kordybach and Smreczak 2003).

1.6.2 Sorption Of Petroleum Hydrocarbons To Humus

The dominant sorbent of hydrophobic compounds in soil is humic acid (Mougin 2002). The two most important forms of sorption of organic contaminants to humic substances are adsorption, defined as adhesion of the chemical to the surface of solid humic material, and chemisorption, the chemical binding to the surface of humic macromolecules (Klavins and Serzane 2000; Mougin 2002). While hydrophobic bonding, hydrogen bonding, charge transfer, ion exchange and ligand exchange are involved in adsorption of hydrophobic organic xenobiotics, van der Waals attraction is believed to be the dominant mechanism. The strongest bonding mechanism between xenobiotic chemicals and humus is believed to be covalent bonding.

Hydrophobicity of humic macromolecules increases with the number of carbon-carbon double bonds and thus with aromaticity, and the affinity of organic contaminants for humic substances is determined by hydrophobicity of the contaminant and humus. Hydrophobic organic chemicals are thus partitioned among various fractions of hydrophobicity in humic material, and the binding efficiency of humic matter dictated by the structure of humic macromolecules (Klavins and Serzane 2000; Mougin 2002). Sorption to humic material greatly reduces bioavailability, and thus toxicity, of organic contaminants to terrestrial and aquatic flora and fauna (Klavins and Serzane 2000; Salanitro 2001). Based upon the sorptive capacity of humic material in compost, the application of 100 to 200 kg compost Mg^{-1} soil dw could bind organic contaminants to soil and thus reduce bioavailability of the compounds to legally acceptable levels

(Verstraete and Devliegher 1996). Humic substances may act as biocatalysts, increasing contaminant decomposition rates in natural environments (Klavins and Serzane 2000).

Through radiocarbon labelling of the aromatic hydrocarbon anthracene and aliphatic hydrocarbon hexadecane, Kästner et al. (1995) found amendment with yard waste compost significantly lowered the extractable fraction of petroleum hydrocarbons. Ninety-two percent of labelled anthracene was recovered by organic solvent and humic acid extraction after 103 days of aerated incubation in non-amended soil, and 8.7% remained irretrievably bound to soil. No mineralisation of labelled anthracene was measured. With 25% w/w compost, 24% of labelled anthracene was mineralised into CO_2, 10% was recovered by extraction and 42% was bound as unextractable residues to soil. Almost 54% of labelled hexadecane in compost-amended soil was mineralised, with 3% recovered by extraction and 15.5% remaining in the soil as unextractable residues. The findings indicated that compost both binds hydrocarbons, rendering them unavailable for extraction, and increases microbial degradation (Kästner et al. 1995).

Further experiments were performed by Kästner and Mahro (1996) on enhancement of PAH degradation through yard waste compost amendment to distinguish between its stimulating effect upon hydrocarbon degrading microorganisms and hydrocarbon sorption onto the organic matter in compost. Sterilised and non-sterilised compost and soil were combined at ratios of 3:1 with naphthalene, phenanthrene, anthracene, fluoranthene and pyrene and incubated for 98 days. Oxygen levels were monitored and the mixtures aerated when required to maintain aerobic conditions. Humic acids were extracted by alkaline hydrolysis to quantify the amount of PAHs remaining bound to the soil matrix after organic solvent extraction. The amount of total PAHs recovered by alkaline hydrolysis from sterilised pure soil stabilised at approximately 20% at the end of incubation, compared to 15% in sterilised soil:sterilised compost mixtures. This difference was attributed to sorption of naphthalene, the most volatile PAH studied, to the humic matter in the sterilised compost mixture.

In pure non-sterilised soil analysed by Kästner and Mahro (1996), fluoranthene and pyrene showed no degradation but phenanthrene and anthracene were completely degraded after 35 days. The PAHs recovered by alkaline hydrolysis, representing sorption to organic matter, increased to a maximum of 30% in the non-sterilised soil. Naphthalene, phenanthrene and anthracene were completely degraded after 20 days in the non-sterilised soil:non-sterilised compost mixture, with complete degradation of fluoranthene and pyrene after 35 days. The measure of PAHs recovered by alkaline hydrolysis increased to 15% shortly after incubation, but all PAHs were completely degraded (as measured by alkaline hydrolysis) by the end of the experiment. The researchers concluded that the effect of compost on PAH depletion in amended soil was the result of real increased microbial degradation in the soil, and not by sorption of PAHs on organic matter or addition of hydrocarbon degrading microorganisms from compost. The increased mineralisation of PAHs observed in compost-amended soil was attributed to co-metabolism or direct aerobic metabolism of the hydrocarbons. The petroleum hydrocarbons tested were fraction 1 and 2 PHCs using the CCME classification system, with no information found on sorption of fraction 3 and 4 hydrocarbons.

Ke et al. (2003) added humic acid and pyrene, a fraction 2 petroleum hydrocarbon, to microcosms of surface sediments from a swamp in Hong Kong. Humic acid was added to raise the sediment organic matter content from 6 to 9%. *Kandelia candel* L. Druce (mangrove) was grown in selected microcosms over the six month trial period. Humic acid amendment significantly increased bacterial populations, measured by MPN counts, in non-vegetated treatments only. Mean pyrene recovery from microcosms non-amended with humic acid was significantly higher in vegetated than in non-vegetated treatments at 70.9 and 61.4%, respectively. In both vegetation treatments, however, pyrene recovery was approximately 37.3% when amended with humic acid. Aerial biomass of *K. candel* seedlings was also significantly lower in microcosms treated with humic acid. These results indicated that the high rate of humic acid applied inhibited plant growth. The lower rate of pyrene removal observed in amended microcosms may have resulted from increased pyrene sorption to humic material or from decreased plant growth.

1.6.3 Stimulation Of Microbial Metabolism Of Petroleum Hydrocarbons

During 98 days of aerobic incubation with naphthalene, phenanthrene, anthracene, fluoranthene and pyrene, no significant changes were observed between microbial populations in non-sterilised and sterilised soil mixed with non-sterilised compost (Kästner and Mahro 1996). Actinomycete and fungi populations in the non-sterilised soil:sterilised compost mixture increased by three orders of magnitude during incubation to numbers similar to those in non-sterilised compost mixtures, however, suggesting the organic material in compost stimulated fungal but not bacterial populations.

Kästner and Mahro (1996) added calcium oxide to increase the pH of Ah soil from 5.2 to 7.0, and compared PAH degradation rates of amended soil with those in non-buffered soil. No significant differences in PAH degradation or extraction were found, demonstrating the pH buffering effect of compost did not influence microbial degradation of hydrocarbons or hydrocarbon fixation to the soil organic matrix within this pH range.

Vouillamoz and Milke (2001) applied diesel fuel to 0.53% w/w concentration to selected soil-compost mixtures of 0, 10, 30 and 60% municipal solid waste (MSW) compost on a wet weight basis. Ammonium nitrate and K_2HPO_4 fertiliser were added to each mixture to yield a C:N:P ratio of 100:5:1, based upon the amount of diesel fuel added, and yields of *Lolium perenne* L. (perennial ryegrass) seeded in selected soil mixtures were measured after 12 weeks of growth in a growth chamber. The results indicated that plant yield increased with compost application rate. Total petroleum hydrocarbon concentrations in unseeded mixtures at all compost concentrations were 600 mg kg^{-1} dw after 12 weeks, while those in seeded soil:compost mixtures measured 300 to 400 mg kg^{-1} TPH dw after 12 weeks. A second experiment conducted at diesel fuel concentrations of 0.23% and 1.24% w/w showed that TPH concentrations (measured as mg kg^{-1} of dry soil:compost) in seeded mixtures declined over time with increasing compost application rate. At both contamination rates compost application at 30% w/w basis was the most effective, reducing TPH concentrations over eight weeks from 2400 to 120 mg kg^{-1} and from 12 100 to 2500 mg kg^{-1}, respectively. The higher final TPH concentrations in the 60% compost mixture relative to the 30% compost mixture suggested that absorption of petroleum hydrocarbons into the MSW compost material may have limited hydrocarbon loss through volatilisation and microbial degradation (Vouillamoz and Milke 2001).

1.6.4 Increased Metabolism Through Improvement In Soil Properties

Application of sewage sludge compost as a bulking agent successfully increased biodegradation of weathered petroleum hydrocarbons in soil from an oil refinery (Brown et al. 1998). Degradation of n-alkanes was also significantly more rapid in soil amended with compost containing high concentrations of N than in compost with low N content.

Loamy soil combined with 5% petroleum refinery sludge w/w was amended with inorganic fertilisers, bacterial inoculum and bulking agents to measure the effect on biodegradation of oil sludge (Vasudevan and Rajaram 2001). The source of the inoculum, comprised of *Acinetobacter*, *Pseudomonas*, *Bacillus*, *Flavobacterium*, *Corynebacterium* and *Aeromonas*, was not identified. Samples were mixed regularly throughout the 90 day incubation to maintain aeration. Amendment with NH_4NO_3, K_2HPO_4 and 10^6 CFU g^{-1} soil bacterial inoculation increased oil degradation from 25% to 66%, with an increase in bacterial population from 5×10^5 to 3×10^{12} CFU g^{-1}. Bacterial inoculation only increased oil degradation to 40% and bacterial population to 2×10^{10} CFU g^{-1}. Compost-amended soil mixtures exhibited oil degradation rates of 28% and bacterial population increases to 9×10^6 CFU g^{-1}. The most successful treatment was bacterial inoculation with addition of wheat bran as a bulking agent, yielding an oil degradation rate of 72% and bacterial population increase to 6×10^{13} CFU g^{-1}. These results were attributed to lowering of soil bulk density and stimulation of microbial metabolism through improved aeration and organic substrate addition (Vasudevan and Rajaram 2001). Application of compost as the sole amendment did not improve bioremediation of oil-contaminated soil in this study, but the design of the experiment made it difficult to compare these results with those of the other treatments tested.

Phenanthrene degradation in sandy loam soil by a microbial consortium adapted to PAHs was studied by Chang et al. (2001) at varying temperatures, pHs, soil water content, PAH concentration and pig waste compost amendment rate. The amount of phenanthrene remaining in sterilised soil, non-sterilised soil and inoculated soil after five days of incubation was 95.0, 82.6 and 6.0%, respectively. Phenanthrene degradation rates decreased with increasing application rate of pig waste compost from 0.055 to 5.50 mg g^{-1} compost, which may have resulted from preferential microbial degradation of organic substrates in compost over phenanthrene. Yeast extract, glucose and pyruvate stimulated phenanthrene degradation in the order listed.

When Palmroth et al. (2002) studied phytoremediation of loamy sand forest soil contaminated with diesel fuel, they found neither 50 mg kg^{-1} NPK fertiliser nor 10 mL kg^{-1} soil MSW compost extract amendment significantly increased PHC mineralisation. These amendments were applied at low rates,,and MSW compost extract application may not improve soil structure and organic matter content the same as MSW compost.

The optimum application rate of compost has been described as that where the positive effect of improvement in soil structure is balanced by a lack of growth limitations due to induction of low O_2 levels in the soil; this rate was defined at 20 to 100 m^3 ha^{-1} by Avnimelech et al. (1990). Using an average bulk density of 0.49 Mg m^{-3} for MSW compost, this rate would be equivalent to 10 to 50 Mg ha^{-1}. Application rates of 25 to 100 Mg ha^{-1} are standard for mature composts (Tester 1989; Dick and McCoy 1993).

1.7 RESEARCH ON FIELD SITES WITH AGED PETROLEUM HYDROCARBON CONTAMINATION

Little research has been done on Canadian sites with historical contamination of petroleum hydrocarbons (Frick et al. 1999; Godwin and Thorpe 2000; McIntyre 2002). Soils with historical or weathered hydrocarbon contamination differ from freshly contaminated soils because the more water-soluble, low molecular weight organic compounds with simple structures have been volatilised or consumed by soil microorganisms, and the remaining contaminants are recalcitrant high molecular weight, structurally complex hydrocarbons which are difficult to biodegrade (Atlas 1981; Song and Bartha 1990; Brown et al. 1998; Carman et al. 1998; Riser-Roberts 1998; Kulakow 2000; Pichtel and Liskanen 2001; Salanitro 2001; McIntyre 2002; Namkoong et al. 2002;

Chaîneau et al. 2003; Reynolds et al. 2003; Sabaté et al. 2004). Fraction 3 and 4 compounds such as alkylated PAHs and cycloalkanes are resistant to microbial attack and thus enriched in aged relative to freshly contaminated soils (Atlas 1981; Riser-Roberts 1998; Kulakow 2000; Chaîneau et al. 2003). In McIntyre's (2002) literature review, two-thirds of biodegradation studies were conducted in laboratories with freshly contaminated soil. All bioremediation field studies on existing oil spills provided oxygenation to soil. This research was designed to evaluate degradation of recalcitrant fraction 3 and 4 PHCs.

Westlake et al. (1978) studied changes in composition of fresh crude oil contamination over three years on boreal soil near Norman Wells, Northwest Territories. Fractionation by liquid chromatography determined oil composition was approximately 8% asphaltenes, 47% saturates, 29% aromatics and 16% polar NSO organic compounds (Jobson et al. 1974). Plots were divided between a cleared cutline and uncleared bush, but the oil treatment killed vegetation on all plots. The asphaltene fraction and aromatic fractions declined slightly, were attributed to experimental error, while the saturate fraction remained at 47% over the period. The NSO fraction increased slightly over two years, but after three years had returned to its original value. Application of 600 kg ha^{-1} N significantly increased soil microbial populations and altered crude oil composition. In fertilised plots asphaltene and aromatic fractions remained stable while the saturate fraction declined sharply, and the NSO fraction steadily increased (Westlake et al. 1978).

Results indicated fertiliser application stimulated microbial degradation of n-alkanes in the saturate fraction, and disappearance of n-alkanes was confirmed by gas chromatography. The relative increase in the NSO fraction was attributed to recovery of partially oxidised compounds, products of co-oxidation of saturate and aromatic compounds, and to decreases in saturate and aromatic fractions. The relative abundance of the aromatic fraction should have increased if aromatic compounds were not being metabolised; this lack of significant change indicated aromatic compounds were being degraded (Westlake et al. 1978). Three years of biodegradation thus resulted in relative enrichment of recalcitrant asphaltenes, aromatic compounds and metabolic products of saturate degradation. Results are consistent with those of Chaîneau et al. (2003), who reported cycloalkanes and linear and branched alkanes in soil freshly contaminated with crude oil were only partially degraded over 480 days in unfertilised biopiles, but completely degraded when inorganic fertiliser was added.

During a 1992 rehabilitation project on Lyell Island in Gwaii Haanas National Park Reserve, Queen Charlotte Islands, British Columbia, heavy PHC contamination was found at a logging site abandoned in 1987 (Lye et al. 1997). Diesel, heating oil and gasoline contaminants had pooled and migrated deeply in the soil to the intertidal zone. Ten thousand m^3 of impacted soil was excavated and placed in a 40 m x 80 m x 3 m lined cell for nutrient amendment, aeration and mixing. Optimisation of O_2 content, nutrients and soil moisture stimulated microbial degradation of total extractable hydrocarbons (TEH) from 13 500 to 1890 mg kg^{-1} within 12 months. The cell was then covered and planted with indigenous vegetation to prevent water infiltration.

In a field trial conducted at Port Hueneme, California, soil with weathered heavy oil and diesel fuel hydrocarbon contamination was excavated into a lined treatment cell and three vegetation treatments applied: no vegetation, a grasses and legumes seed mix, and a native grasses mix (Banks et al. 2003). Seeding rates were not specified; inorganic fertiliser and irrigation were applied. After 29 months, TPH concentrations decreased from 3000 mg kg^{-1} to 1900 mg kg^{-1} in non-vegetated treatments. This decrease was attributed to mixing and O_2 exposure during movement of contaminated soil into the treatment cell. Total petroleum hydrocarbons in vegetated treatments were 1300 and 1450 mg kg^{-1} for the grasses and legumes seed mix and native grasses seed mix, respectively, yielding a significant difference between vegetated and non-vegetated treatments.

1.7.1 Microbial Populations In Soil With Weathered Contamination

As the recalcitrance of organic compounds remaining in contaminated soils increases with time, soil microbial community diversity becomes more important. Because the many compounds present in petroleum hydrocarbons are mineralised by different microorganisms, reductions in contamination levels increase with species richness in the soil (Heinonsalo et al. 2000; Reynolds et al. 2003). Plants increase this diversity by producing root exudates that attract soil microorganisms, and biostimulation provided by plants increases in importance with the recalcitrance (and thus age) of the hydrocarbon contamination (Heinonsalo et al. 2000; Reynolds et al. 2003; White et al. 2003).

Few studies have indentified microbial genera or species in weathered soils, due in part to the low survival of hydrocarbon-degrading bacteria on plate cultures (Balba et al. 1998; Semenov et al. 1998; Trevors 1998; White et al. 1998; Alexander 1999; RTDF 1999). Saul et al. (2005), however, reported that hydrocarbon-degrading microorganisms had greater survival on plate cultures than did the total heterotrophic population. Plate counting typically detects less than 10% of the total microbial community in a sampled soil (Zuberer 1994; Trevors 1998; Alexander 1999; Van Hamme et al. 2003; Saul et al. 2005). Semenov et al. (1998) reported that plate counting of fungal species *Candida lipolytica* and *Cladosporium resinae* immediately after inoculation into oil-contaminated soil yielded approximately 20 and 40%, respectively of the initial dosage. These losses upon microbial inoculation were attributed to formation of cell aggregates, adhesion of cells to soil particles, and mortality of cells and spores (Semenov et al. 1998).

Siciliano et al. (2003) studied phytoremediation of soil with weathered diesel fuel and heavy oil contamination over an 18-month field trial, with inorganic fertiliser application. Fifteen bacterial species were identified in contaminated soil using denaturing gradient gel electrophoresis of amplified 16S ribosomal RNA genes, with no significant difference between vegetated and non-vegetated soils. Species were not completely identified, nor was the cause of this low microbial diversity discussed. The total heterotrophic population in vegetated and non-vegetated treatments remained steady at 4×10^8 and 7×10^7 CFU g^{-1} soil, respectively. The combined prevalence of the catabolic genes alkane monooxygenase (*alkB*), naphthalene dioxygenase (*ndoB*) and catechol-2,3-dioxygenase (*xylE*) declined from 35 to 18% in both vegetation treatments, while that in non-vegetated soil remained at 21%. This shift in the microbial community corresponded to PHC degradation measured in soil (Siciliano et al. 2003), but no explanation for the change in catabolic gene prevalence was provided. MPN analysis of petroleum degrading microorganisms, conducted from 18 to 29 months in the study, demonstrated significantly higher petroleum degraders in vegetated than non-vegetated soil treatments (Banks et al. 2003).

Populations of aerobic, iron-reducing, denitrifying and sulphate-reducing bacteria were sampled to 70 cm depth in boreal forest soils in northern Alberta contaminated with diesel fuel ten years earlier (McIntyre 2002). At Pony Creek, 30 cm of loam overlay sandy clay soil with 19.2% moisture content; at Birch Mountain, the sandy loam soil had 14.5% moisture content. Effects of contamination and NPK enrichment were weaker at Birch Mountain, where temperatures and precipitation were lower. Effects on aerobic and anaerobic microorganisms were not consistent with soil depth. Bacteria tentatively identified in contaminated Pony Creek soil were *Achromobacter*, *Alcaligenes*, *Flavobacterium* and *Pseudomonas*, all previously identified as hydrocarbon utilisers. Diesel fuel contamination on non-fertilised plots and application of NPK fertiliser to non-contaminated control plots did not significantly affect aerobic microbial population size at Pony Creek or Birch Mountain, at 3.09×10^5 and 3.63×10^5 CFU g^{-1} dry soil, respectively, on control non-fertilised plots and across soil depths. Fertiliser application to contaminated plots at Pony Creek increased aerobic microbial populations by 16%, but not at Birch Mountain soils.

Denitrifying microorganisms at Pony Creek were 148% higher in contaminated than control plots, where populations were 4.07×10^0 MPN g^{-1} across soil depths (McIntyre 2002). Denitrifying microorganisms were 285% higher on fertilised than non-fertilised plots at Pony Creek. Contamination on non-fertilised plots and fertiliser application to control plots did not significantly affect denitrifying microbial populations at Birch Mountain, with 3.63×10^0 MPN g^{-1} on control non-fertilised plots. Fertiliser application to contaminated plots increased denitrifying populations by 166% at Birch Mountain.

Iron-reducing microorganisms on non-fertilised soil at Pony Creek and Birch Mountain, at 9.33×10^4 and at 7.41×10^4 MPN g^{-1}, respectively, across soil depths, did not differ significantly with fertiliser or contamination treatment (McIntyre 2002). Fertiliser application to contaminated plots at Birch Mountain increased iron-reducing bacteria by 18% compared to non-fertilised contaminated plots (not statistically significant); fertiliser treatment did not significantly affect population size in contaminated soils at Pony Creek. Fertiliser application to controls at Pony Creek increased sulphate-reducing bacteria at

4.07×10^4 MPN g^{-1}, across soil depths, by 18%. The population size at Birch Mountain, at 6.31×10^4 MPN g^{-1}, was not significantly affected by fertiliser application only. Contamination reduced sulphate-reducing microbial populations at both sites by 18%. Increases in sulphate-reducing soil microorganisms after fertiliser application to contaminated soils at both sites were not statistically significant (McIntyre 2002).

Atagana et al. (2003) studied microbial degradation of PAHs in soil with weathered creosote contamination in a soil microcosm experiment. Carbon to nitrogen ratios were adjusted to values between 25:1 and 5:1, with non-amended controls. Phenols and naphthalene, a fraction 1 PHC, were completely degraded after 6 weeks. The fraction 2 hydrocarbons anthracene and phenanthrene were slower to degrade; phenanthrene proved much more susceptible to microbial attack than anthracene, attributed to its higher solubility (1.1 and 0.05 mg L^{-1}, respectively). Degradation rates were slowest for fraction 3 PHCs chrysene and benzo(a)pyrene, with incomplete degradation after 6 weeks of incubation. Nitrogen amendment increased degradation rates for all three fractions of hydrocarbons, with optimal results for all hydrocarbons at C:N of 25:1, but the published results did not indicate what compound degradation rates were at each C:N treatment.

1.8 SUMMARY AND CONTRIBUTIONS OF PROPOSED RESEARCH

1.8.1 Summary

Microbial metabolism is the dominant form of degradation of petroleum hydrocarbons in the natural environment. For large-scale contamination, bioremediation technology is best suited to remove these soil contaminants. Microbial activity is stimulated by plant establishment, and phytoremediation has been an effective method to increase microbial degradation of petroleum hydrocarbons in soil. Methods to further increase biodegradation of organic contaminants are being sought, as these compounds are often hydrophobic and have low bioavailability to microorganisms and plants. Biodegradation of recalcitrant compounds, however, is often neglected in bioremediation studies.

Compost adds organic matter to soil and thus improves soil porosity and water holding capacity. These improvements, as well as the addition of organic matter itself, increases microbial populations, metabolic activity and plant growth in amended soils. Compost may supply nutrients to soil microorganisms and plants, and humic matter in compost may sorb organic contaminants rendering them unavailable for uptake by soil flora and fauna. These effects can be long-lasting and may improve microbial degradation of petroleum hydrocarbon contamination.

Little research has been done to evaluate effects of compost application to recalcitrant petroleum hydrocarbon contamination on a field scale. The research proposed here will address these gaps in current knowledge about petroleum hydrocarbon degradation.

1.8.2 Contribution of Proposed Research

Degradation of petroleum hydrocarbon contaminants, in particular fraction 3 compounds, can be slow in northern climates and difficulty is often encountered in meeting legislative requirements. Government regulations are growing stricter, and industry is increasingly required to remediate sites where abandonment was permitted in the past. The sheer volume of contaminated soil is too large to make excavation and disposal, the method currently preferred by industry, a viable long-term solution. Bioremediation is increasingly used to treat contamination of this type. Methods must be found to stimulate biodegradation of petroleum hydrocarbon contamination, and bioremediation and compost application may prove to be effective technologies.

There is a large gap in knowledge of the potential for compost as an amendment in bioremediation, particularly bioremediation of weathered hydrocarbon contamination. Most studies have focused on remediation of TPH or fraction 1 and 2 petroleum hydrocarbons, using freshly contaminated soil, and have been conducted on a small scale (Salanitro 2001). The mechanisms by which compost can stimulate microbial degradation of contaminants requires further study. Davis et al. (2003) found that laboratory studies of bioremediation of creosote contamination could not be accurately extrapolated to large-scale application, so a field trial is required to accurately assess the feasibility of bioremediation and phytoremediation technology for industrial sites.

Most studies on microbial metabolism of petroleum hydrocarbons have been limited to laboratory experiments. These studies are confined to degradation of one specific hydrocarbon or group of hydrocarbons, and utilise a pure culture of a specific microbial species or a consortium of microorganisms isolated from soils (Trevors 1998; Salanitro 2001). From these studies comes our current understanding of the relative abundance of hydrocarbon-degrading microorganisms in pristine and contaminated environments, metabolic pathways, hydrocarbon toxicity to soil microorganisms, intermediate and metabolite production, and the biodegrading ability of bacteria, fungi, actinomycetes and algae species (Salanitro 2001). Rates of metabolic activity for pure cultures cannot predict performance of a diverse microbial community, nor can degradability of a petroleum hydrocarbon mixture be predicted from biodegradation studies of components in isolation. Biodegradation rates in culture conditions in the laboratory will not correspond to microbial performance in the more hostile environment of polluted soils (Salanitro 2001). Analysis of soil from a field trial is required to assess the effect of oil contamination, compost and fertiliser amendments, and vegetation establishment on soil microbial community structure and function.

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2. MICROBIAL POPULATION DYNAMICS IN HYDROCARBON CONTAMINATED SOIL

2.1 INTRODUCTION

Studies in a variety of soils and climates have demonstrated that indigenous soil microorganisms can remediate low to moderate levels of hydrocarbon contamination (Lye et al. 1997; Riser-Roberts 1998; Frick et al. 1999; Stanley et al. 2000; Andreotti et al. 2001; Mougin 2002; Rahman et al. 2002; Germida and Farrell 2003). Although hydrocarbon contamination can increase soil microbial populations (Nichols et al. 1997; Margesin et al. 2000a; Salanitro 2001; McIntyre 2002; Palmroth et al. 2002; Chaîneau et al. 2003; Lors et al. 2004; Salminen et al. 2004), microbial activity can be inhibited by toxicity of the hydrocarbons, reduction in soil aeration by filling in of soil pores, induced soil hydrophobicity, and alteration in the balance of inorganic nutrients resulting in induced deficiencies of nitrogen (N) and phosphorus (P) (Westlake et al. 1978; Atlas 1981; Lye et al. 1997; Riser-Roberts 1998; Chang et al. 2001; Hutchinson et al. 2001a; Salanitro 2001; McIntyre 2002; Maliszewska-Kordybach and Smreczak 2003). Organic amendments such as compost may sorb contaminants and improve soil physical and chemical properties, including soil aeration, and may thus increase microbial activity and aid in vegetation establishment (Dick and McCoy 1993; Hill and James 1998; Stratton et al. 1998; Liem et al. 2003).

While a large body of research has been compiled in the area of bioremediation and phytoremediation of organic chemicals, there remains a lack of information on the application of bioremediation to petroleum hydrocarbon (PHC) spills on soil in Alberta. Few studies have been conducted to date in Alberta, and most phytoremediation studies have employed agronomic species that may not be compatible with the revegetation objectives of industry and government. The potential for compost as a soil amendment to enhance phytoremediation has also not been investigated. This simulated in situ study was designed to answer these questions. Because soil microbial activity is increased by addition of nutrient, water, oxygen (O_2) and organic matter, the effect of compost on these soil physical and chemical properties was measured directly as an estimate of bioremediation potential from compost treatment. Changes in population size and activity in the soil microbial community were studied separately.

2.2 RESEARCH OBJECTIVES AND HYPOTHESES

2.2.1 Research Objectives

- To determine the effects of crude oil contamination on soil microbial community composition, population size and metabolic activity.
- To determine the effects of compost amendment on soil microbial community composition, population size and metabolic activity in contaminated and non-contaminated (control) soils.
- To determine the effects of inorganic fertiliser amendment on soil microbial community composition, population size and metabolic activity in contaminated and non-contaminated soils..
- To examine the correlation, if any, between microbiological parameters and PHC loss from contaminated soils.
- To examine how soil microbial community composition, population size and metabolic activity varies with soil depth and soil conditions.

2.2.2 Working Hypotheses

- There will be significant differences in microbiological parameters between non-contaminated soil and soil contaminated with crude oil.
- There will be significant differences in microbiological parameters between soil amended with compost and non-amended soil.
- There will be significant differences in microbiological parameters between soil with carbon to nitrogen (C:N) ratio set to 10:1, adjusted with ammonium sulphate ($(NH_4)_2SO_4$) fertiliser, and non-adjusted soil without inorganic fertiliser addition.
- There will be significant differences in microbiological parameters between contaminated soil with and without compost amendment.

- There will be significant differences in microbiological parameters between contaminated soil without amendment and contaminated soil with C:N ratio set to 10 (adjusted with $(NH_4)_2SO_4$ fertiliser).
- There will be significant differences in microbiological parameters with soil depth.
- There will be correlations among microbiological and soil parameters including pH, O_2 concentration, total extractable hydrocarbon (TEH) concentration, PHC concentrations by Canadian Council of Ministers of the Environment (CCME) fraction (CCME 2001), organic matter, salinity as measured by electrical conductivity (EC), ammonium (NH_4^+) concentration, nitrate (NO_3^-) concentration, manganese (Mn^{+2}) concentration, sulphate (SO_4^{-2}) concentration, total N concentration, C:N ratio and moisture content.

2.3 MATERIALS AND METHODS

2.3.1 Study Area

The field research site was located at the City of Edmonton Waste Management Centre in Edmonton, Alberta, Canada. Edmonton is located in the Boreal Plains ecozone, with a dry subhumid continental climate. Mean monthly temperatures in Edmonton vary between -16.6 and 22.2 °C and the mean annual precipitation is 466 mm (Environment Canada 2004) (Table A3).

2.3.2 Experimental Design and Treatments

2.3.2.1 Scope

This study investigated bioremediation of petroleum contaminated soil at an experimental site in Edmonton, Alberta from June 2003 through August 2004. Microbial population size, composition and structure, monitored over the course of the experiment, were compared between the following four factors.

- Non-contaminated soil to soil contaminated with light crude oil.

- Soil amended with compost at 20% dry soil mass basis (dw) (10% wet soil volume basis) to non-amended soil.
- Soil with C:N adjusted to 10:1 using $(NH_4)_2SO_4$ fertiliser (21-0-0-24) to soil without nutrient amendment.
- Soil samples collected at 0 to 10 cm depth and at 10 to 20 cm depth.

2.3.2.2 Microbiological parameters

The following main response variables, the microbiological parameters, were measured at the end of the field experiment.

- Size of viable soil fungal populations measured by plate counting as described in Appendix A of the United States Department of Agriculture (USDA) Remediation Technologies Development Forum (RTDF) Phytoremediation Action Team field protocol (Parkinson 1994; RTDF 1999).
- Size of culturable soil aerobic heterotrophic bacteria (and actinomycetes if culturable) populations measured by plate counting on agar.
- Size of soil aerobic microbial population capable of degrading aliphatic petroleum hydrocarbons measured by most probable number (MPN) technique in 96-well plates using hexadecane.
- Size of soil aerobic microbial population capable of degrading aromatic petroleum hydrocarbons measured by MPN technique in 96-well plates using polycyclic aromatic hydrocarbons (PAHs) in pentane solution.
- Size of soil microbial biomass using chloroform fumigation technique (Horwath and Paul 1994).
- Aerobic respiration rate measured by carbon dioxide (CO_2) evolution through incubation and gas chromatography.
- Aerobic respiratory ratio, calculated as moles CO_2 produced/moles O_2 consumed.
- Methanogenic respiration rate measured by methane (CH_4) evolution through incubation and gas chromatography.
- Colony morphological richness estimated from aerobic heterotrophic plate counts on agar at 10^{-5} soil dilution.

- Shannon-Weaver colony morphological diversity index estimated from aerobic heterotrophic plate counts on agar at 10^{-5} soil dilution.

2.3.2.3 Independent variables

The experimental independent variables were as follows.

- Soil contamination: Soil was spiked to 10 000 mg kg^{-1} TEH with light crude oil from the Edmonton ESSO Strathcona refinery and non-contaminated soil used as a control.
- Vegetation cover: A non-seeded treatment was used for microbiological sampling.
- Availability of N: Nitrogen supply to plants and microorganisms was measured by C:N ratio in the soil. The two treatments assessed were the unadjusted concentrations in the soil (soil without inorganic fertiliser amendment), and soil with C:N adjusted to 10:1 by $(NH_4)_2SO_4$ application.
- Compost application: Mature municipal solid waste/biosolids co-compost from the Edmonton Waste Management Centre was applied at rates of 0 or 20% dw.

2.3.2.4 Fixed conditions

To compare treatments, the following conditions were fixed.

- Soil hydrocarbon concentrations between compost treatments and inorganic fertiliser treatments. The control treatment was non-contaminated soil.
- Seed mix treatment. Three seed mixes were tested by Graham (2005), but the non-seeded control treatment was the only vegetation treatment sampled for this study.

2.3.2.5 Response and extraneous variables

The main response variables were the microbiological parameters listed above. The following response variables were also measured.

- Soil concentrations of hydrocarbons (TEH) measured at regular time intervals.
- Soil physical and chemical properties, including pH, EC, organic matter content, nutrient concentrations, C:N ratio and concentrations of electron acceptors SO_4^{-2}, NO_3^-, Mn^{+2} and NH_4^+.
- Oxygen concentration in soil using a probe.
- Soil moisture content at the time of sampling.

Extraneous variables expected and encountered were as follows.

- Weather conditions, particularly precipitation and temperature.
- Invasion by weeds.

2.3.2.6 Experimental design

To ensure experimental control, experimental units consisted of 20 L (28.5 cm diameter, 40 cm depth) plastic pails with an internal drainage system to remove leachate after each rainfall event. This internal drainage system was comprised of a 5 cm layer of sterilised sand at the bottom of each pail and 1.9 cm diameter perforated PVC tubing, through which leachate was pumped from the bottom of each pail to prevent flooding (Figure 2.1). Volume of leachate collected from each pail was recorded (Graham 2005). Weed invasion was monitored but not controlled, and weeds such as *Erodium cicutarium* (L.) L'Hér. ex Ait. (redstem stork's bill), *Kochia scoparia* (L.) Schrad. Mexican fireweed, *Hordeum jubatum* L. (foxtail barley) and *Crepis tectorum* L. (narrowleaf hawksbeard) dominated canopy cover by May 2004 (Table A4) (Graham 2004).

Non-contaminated topsoil (40%) and subsoil (60%) from the Edmonton Waste Management Centre were combined on a wet mass basis to form the non-contaminated control soil used in the experiment. The resulting sandy loam soil contained 73% sand, 14% silt and 13% clay, with low total organic carbon (TOC) content of 1.8%. Crude oil, compost and inorganic fertiliser amendments were thoroughly mixed in a cement mixer to create a homogenous soil. Soils were settled into the pails by gently tapping the bottom of the pail on the ground, resulting in depths of approximately 25 cm; pails were buried in the ground to a depth of 35 cm to simulate in situ field conditions (Graham 2005).

Mature compost prepared by co-composting municipal solid waste and biosolids was obtained from the City of Edmonton (Table 2.1). The material is class B compost according to CCME quality guidelines (CCME 1995) due to elevated concentrations of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) (Table A5).

The two compost treatments tested were amended at 0 and 20% dw (10% wet soil volume basis). This application rate was selected by Graham (2005) to increase the TOC level in the treatment soil to background levels for the area. Fertiliser application was designed to optimise nutrient levels for microbial degradation of hydrocarbons. A C:N ratio of 10:1 was considered ideal for microbial populations (Huesemann 1994), and $(NH_4)_2SO_4$ was incorporated at a rate to achieve a hydrocarbon C to N ratio of ~10 (Graham 2005). Due to the lower amount of soil in the compost + fertiliser treatment, 60 g of $(NH_4)_2SO_4$ was added, compared to 96 g added to the fertiliser only treatment. The resulting C:N ratios in the fertiliser and compost + fertiliser treatments were 11.1 and 11.8 in the contaminated soil and 8.6 and 12.1 in the control soil, respectively. No adjustment to C:N was made (no inorganic fertiliser added) for the control (no fertiliser). To maintain equal pail volumes, fertiliser and unamended pails contained 23.2 kg of soil dw and compost and compost-fertiliser pails contained 14.9 kg soil dw (Graham 2005).

2.3.3 Soil and Vegetation Sampling

2.3.3.1 Sampling for treatment characterisation and hydrocarbon degradation

Vegetation, soil physical and chemical properties, with the exception of oxygen concentration, and hydrocarbon concentrations were measured and analysed by Graham (2005) as part of her MSc research. The results are used in this thesis to characterise the conditions under which microbial populations developed and functioned.

Soil samples were collected at the beginning (June 2003) and end (August 2004) of the study. Three composite samples of topsoil, subsoil and compost were collected to characterise initial materials in 2003 before soils were placed in pails. Samples were collected from 0 to 10 and 10 to 20 cm depths in 2004 (Graham 2004; Graham 2005).

Vegetation assessments were conducted in September 2003 and May and August 2004 to capture early and late season species. Total canopy cover (%) and species composition were determined at each time. Destructive vegetation sampling for above ground and root biomass was conducted at the end of the experiment in August 2004.

2.3.3.2 Soil sampling for microbiological parameters

On August 26 and 27, 2004, three soil cores were collected from each pail to 20 cm depth using a soil corer of 1.9 cm diameter. Soil cores were taken from randomly chosen locations on the soil surface to avoid plants and to minimise disruption for Graham's sampling. Each core was divided into 0 to 10 and 10 to 20 cm depth increments. The three cores per pail were combined into one composite sample for each depth increment for each pail. Each composite sample was stored in labelled double-wrapped Ziploc™ bags. Each pail was one replicate and each treatment was replicated four times in the random block experimental design, yielding four samples per sampling depth for each soil treatment. These four samples could not be composited for analysis because there was no estimate of the variability between blocks (Wollum 1994). Samples were collected from non-contaminated, non-amended pails first and contaminated, amended pails last to minimise any residual contamination from the soil corer. The corer was washed with 5% v/v bleach solution, rinsed twice with tap water, and rinsed once with distilled water between samples to prevent contamination (Wollum 1994; Zuberer 1994). The O_2 concentration in each pail was measured at 5 and 15 cm depths with a Demista OT-21 oxygen probe provided by the City of Edmonton.

2.3.4 Soil Laboratory Analyses

2.3.4.1 Physical and chemical properties

Samples collected by Graham in June 2003 and August 2004 were analyzed for TEH between C_{11} and C_{60}, trace metals, fecal coliform, *Salmonella*, TOC, total Kjeldhal nitrogen (TKN), NH_4^+, NO_3^-, pH, electrical conductivity (EC), sodium adsorption ratio (SAR), C:N ratio, moisture content, base saturation, calcium (Ca^{+2}), potassium (K^+), magnesium (Mg^{+2}) and sodium (Na^+). Individual carbon (C) lengths were summed from the TEH analysis to correspond with fraction 2 ($>C_{10-16}$), fraction 3 ($>C_{16-34}$) and fraction 4 ($>C_{34}$) hydrocarbons (CCME 2001). Analyses for bulk density, SO_4^{-2} and Mn^{+2} were added in August 2004 (Graham 2005).

2.3.4.2 Sample preparation for microbiological analysis

Soils were sieved through a 2 mm sieve in the laboratory to remove gravel and standardise soil texture (Wollum 1994; Stotzky 1997). Rocks and other material which could not pass through the sieve were saved separately. Many samples were too wet to be sieved without great difficulty. Dilutions of soils too wet to be sieved were prepared by random selection of soil from the unsieved samples to form a consolidated sample. The sieve was sterilised by rinsing with distilled water and flaming between samples.

Plastic bags are permeable to CO_2 and O_2 but not to water vapour, thus maintaining aerobic and soil moisture conditions in the sample during storage. Samples were plated within three days of collection, minimising storage time and exposure of anaerobic microorganisms to O_2 (Wollum 1994; Trevors 1998). Soil microbial biomass and respiration measurements were conducted one and two weeks later, respectively. The samples were kept in a cooler with ice packs during the first three days following collection, and in a cold storage room at 4 °C afterwards. Each sample was approximately 125 g wet weight. Soil remaining after biomass, respiration and moisture content analyses was air-dried and saved for possible further analyses.

2.3.5 Microbiological Analyses

2.3.5.1 Preparation of dilutant

Buffer and salt solutions are used for diluting soil samples to prevent osmotic shock to cells (Zuberer 1994). Buffer solution for soil dilutions was prepared in a 2 L Erlenmeyer flask with 0.65 g dipotassium hydrogen phosphate (K_2HPO_4), 0.35 g potassium dihydrogen phosphate (KH_2PO_4) and 0.10 g magnesium sulphate ($MgSO_4 \cdot 7H_2O$) in distilled water to prepare 1 L of dilutant (Margesin and Schinner 1997; RTDF 1999). The dilution solution was adjusted to pH 7.2, as measured by a pH probe, with 0.1 N hydrochloric acid (HCl) or 0.1 N sodium hydroxide (NaOH) as required (Jobson 2004). Blanks for the initial (10^{-1}) dilution were prepared by adding 90 mL of dilutant to one 500 mL glass dilution bottle for each soil sample. Blanks for the remaining dilutions were prepared by adding 9 mL of dilutant to each of six 16 mm test tubes. The same dilution blanks were used for Petri dish plating and 96-well plating procedures (Jobson 2004).

The dilution blanks were sterilised before soil addition by autoclaving for 30 minutes at 121 °C and 103.4 kPa (Zuberer 1994). Evaporative loss due to autoclaving has been estimated at 3% maximum and its effect upon solution concentration thus insignificant (Zuberer 1994). Evaporative loss from these large dilution bottles was significant and calculation of dilution concentrations adjusted for the loss of volume during autoclaving.

2.3.5.1 Preparation of soil dilutions

Seven levels of soil dilution were prepared for each soil sample in the series 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and 10^{-7}. Bottles of 500 mL volume were used for the initial 10^{-1} dilution blanks, and 16 mm test tubes were used for the remaining blanks. Ten g of soil (wet weight) were taken from each sample and weighed into the 10^{-1} dilution blank. This blank was capped and shaken vigorously by hand, with twelve shakes per blank, to thoroughly mix soil in the dilutant and to break up soil aggregates (Zuberer 1994). Physical dispersion of soil solutions by shaking yields little difference in numbers of bacteria extracted compared to ultrasonification, centrifugation and chemical dispersal, which can damage microbial cells (Trevors 1998). The resulting soil suspension was diluted by a factor of 10, creating a 10^{-1} soil dilution. The next dilution level was prepared by pipetting 1 mL of 10^{-1} soil dilution into the 10^{-2} blank. The resulting 10^{-2} soil solution in the test tube was mixed using a vortex (Jobson 2004). This procedure was repeated to create seven soil dilutions (Zuberer 1994; Jobson 2004).

The oven-dry soil moisture content, measured by drying at 105 °C overnight, was used to calculate the mass of dry soil in the 10 g of wet soil added to the first dilution blank. The exact level of dilution was then calculated, and population estimates corrected by multiplying by the ratio of actual dilution to intended dilution (Woomer 1994).

An experiment was run in 2005 to determine if preparation of the initial dilution solution was sufficient to disperse soil clumps (refer to section 2.3.7.1). A homogenised sample of non-contaminated, non-amended soil was diluted to 10^{1} as done previously and 10^{2} to 10^{7}

dilutions prepared after 12, 24, 36, 48, 60 and 72 shakes of this solution. Solutions of dilution were plated on plate count agar (PCA) and plates incubated and counted as described previously.

2.3.5.3 Inoculation of petri dishes

Two media were used for dilution plate counting: Difco PCA for enumeration of aerobic heterotrophic bacteria and Martin's medium for enumeration of viable fungi (RTDF 1999). Compared to pour plating, the spread plate technique produces higher colony counts and improves visual differentiation between colonies of bacteria and actinomycetes (Zuberer 1994). Four replicate Petri dishes were used for each dilution of each sample on each media type. The plates were inoculated with 0.1 mL of soil dilution one level higher than the level desired, and these 0.1 mL volumes were spread on agar surfaces with a bent sterile glass rod. For example, 0.1 mL of 10^{-2} soil dilution was spread to create a plate at 10^{-3} dilution. Martin's media plates for viable fungi enumeration were inoculated with dilution series 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} only, while PCA plates for total aerobic bacteria and actinomycetes enumeration were inoculated with the full dilution series (to 10^{-8}) excluding 10^{-1} (Nichols et al. 1997). The rod was sterilised with 95% ethanol and flaming between each group of plates (Zuberer 1994; Jobson 2004). Plates were stacked lid-down, wrapped in plastic sleeves and incubated in darkness at room temperature for 10 days. Population estimates were derived from the dilution yielding the highest count between 30 and 300 colonies per plate (Zuberer 1994).

2.3.5.4 Colony morphological richness and diversity

The richness and diversity of the soil bacterial community were estimated from counts of aerobic heterotrophic microbial plate counts on PCA. Bacterial colonies at 10^{-5} soil dilution were differentiated by colour, shape, and surface patterns upon visual inspection. The number of colony types per plate was recorded as colony morphological richness, and the numbers of individuals of each colony type were used to calculate colony morphological diversity using the Shannon-Weaver diversity index: $H = -\Sigma P_i \ln P_i$ where P_i = proportion of total colonies of colony type i (Townsend et al. 2000).

2.3.5.5 Inoculation of 96-well plates

The MPN technique is used to measure heterogeneous microbial populations based upon a common attribute, such as hydrocarbon utilisation (Woomer 1994). Five replicate wells were used for each dilution of each sample on each well plate. These rows of wells were labelled with the soil dilution level to be counted: 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} (Nichols et al. 1997). The wells were inoculated with 20 μL of the appropriate soil dilution. One row was not inoculated to serve as a sterile negative control, and two columns were inoculated with a known hydrocarbon-degrading microbial consortium to serve as a positive control (Walker and Colwell 1976; Haines et al. 1996; Foght 2004).

The sheen screen method was designed for rapid enumeration of several hundred samples (Brown and Braddock 1990). The 96-well microtiter plate procedure described below is an adaptation of the 24-well sheen screen assay, and the two methods yield equal population estimates (Haines et al. 1996). The sheen screen method, where emulsification of the oil sheen on wells indicates a positive score, cannot be used on 96-well plates because wells are too small to detect it (Haines et al. 1996). Both methods underestimate population density of hydrocarbon-degrading microorganisms. While one cell is theoretically required to produce a positive result, Haines et al. (1996) reported that one to nine cells per well appear to be required. MPN methods remain the most accurate for enumerating hydrocarbon-degrading microorganisms (Wrenn and Venosa 1996) because non-hydrocarbon-utilising microorganisms may grow on impurities in solid media (Walker and Colwell 1976; Haines et al. 1996). A MPN calculator was used to translate 96-well plate data into population estimates (Curiale 2000), as per Johnsen et al. (2002).

Degradation of aliphatic hydrocarbons and aromatic hydrocarbons was measured separately. In plates designated for enumeration of aromatic hydrocarbon degradation, 10 μL of filter-sterilised PAH mixture were deposited in each well before pouring of growth media. The PAH mixture was 10 g L^{-1} phenanthrene, 1 g L^{-1} pyrene, 1 g L^{-1} fluorene and 1 g L^{-1} dibenzothiophene in pentane (Kästner et al. 1994; Wrenn and Venosa 1996). Pentane evaporates rapidly and deposits PAHs onto the surfaces inside each well (Wrenn and Venosa 1996). Plates designated for enumeration of aliphatic hydrocarbon degradation were treated with 5 µL of filter-sterilised hexadecane per well after pouring of growth media but before inoculation (Wrenn and Venosa 1996).

Fifty microliters of sterile 3 g L^{-1} solution of the indicator iodonitrotetrazolium violet (INT) ($C_{19}H_{13}N_5O_2ICl$) was added to each well after the incubation period. Formation of a red or pink precipitate, indicating INT reduction due to respiratory electron-transport activity of aerobic microorganisms, confirmed presence of active hydrocarbon-degrading microorganisms. Products of partial PAH oxidation, ranging in colour from bright yellow to brown, were also used to identify positive wells where INT reduction was less effective (Wrenn and Venosa 1996; Johnsen et al. 2002). Wells were scored after overnight incubation at room temperature with INT (Haines et al. 1996; Wrenn and Venosa 1996; Johnsen et al. 2002).

Following well scoring, 25 µL of an electron donor solution was added to each well of the PAH enumeration plates to increase INT assay sensitivity (Johnsen et al. 2002). The electron donor solution was a 33.2 mM solution of glucose, pyruvate and succinate (each) in 40 mM Tris buffer adjusted to pH 6.5. Wells were scored again after 90 minutes of incubation (Johnsen et al. 2002).

2.3.5.6 Microbial biomass estimation by chloroform fumigation incubation method

Microbial biomass accounts for 1 to 3% of soil organic carbon (C), but drives cycling of soil C and nutrients (Horwath and Paul 1994; Martens 1995). Chloroform fumigation is the most widely used method for estimation of C contained in soil as microbial biomass, and is the standard by which other methods are calibrated (Martens 1995). Microbial biomass is estimated by directly extracting and analysing cell components released when soil microorganisms are lysed by chloroform (Horwath and Paul 1994; Jørgensen 1995). Glucose-induced respiration is also used to determine microbial biomass, but will apply only to microorganisms utilising glucose (Margesin et al. 2000b). Margesin et al. (2000b) reported significant correlations of 0.5 with extractable hydrocarbons and microbial biomass C and CO_2 respiration. Fumigation periods of one day are conducted for rapid measurement, but up to five days may be required (Horwath and Paul 1994).

The sieved soil samples were stored in a cooler with ice packs for a maximum of three days before biomass measurement (Horwath and Paul 1994). All biomass measurements were conducted in a fume hood. Each sample was split into two 15 g wet weight sub-samples, one to be fumigated. Each sub-sample was placed in a 125 mL bottle. The sub-sample to be fumigated was placed in a dessicator lined with wet tissue paper to minimise soil moisture loss. A beaker containing 30 to 35 mL ethanol-free chloroform and a few boiling chips was also placed in the dessicator. The desiccator was evacuated until the chloroform boiled. The chloroform was boiled for two minutes, the valve on the desiccator closed, and the desiccator left in darkness at ambient temperature for 24 hours (Mueller et al. 1992; Jørgensen 1995). The non-fumigated control sub-samples were weighed and extracted on the same day (Horwath and Paul 1994).

After incubation, the desiccator was evacuated five times at two minutes each to remove residual chloroform. Each sub-sample was extracted with 50 mL of 0.25 M dipotassium sulphate solution (K_2SO_4) for 30 minutes in an oscillating shaker at 200 revolutions minute^{-1}. The ratio of K_2SO_4 extractant to soil dry weight was 5:1 volume/weight. This ratio must be increased in soils containing more than 20% organic matter (Jørgensen 1995), but organic matter in soil samples from this experiment was not expected to exceed this value (Graham 2004). The high K concentration in the extractant prevents adsorption of the NH_4^+ released by fumigation, and inhibits microbial decomposition of cell material extracted after fumigation. The extractants were filtered through Fisherbrand P2 filter paper (fine porosity and slow flow rate) and stored at 4 °C prior to analysis on a C analyser. A blank sample containing extractant alone was analysed with each batch of samples, and three samples per batch were duplicated to provide controls (Horwath and Paul 1994; Jørgensen 1995).

2.3.5.7 Microbial activity estimation by respiration analysis

Carbon respiration, energy production from the breakdown of organic compounds, is the most important redox reaction of metabolism. Respiration is a measure of the total biological activity in soil resulting from mineralisation of organic matter, or specifically resulting in CO_2 production (Wollum 1994; Margesin et al. 2000a). Aerobic respiration, the dominant form of respiration among hydrocarbon-degrading microorganisms, consumes one mole of O_2 for each mole of CO_2 produced: $O_2 + C \rightarrow CO_2$ standard reduction potential $E'_O = +0.82$ V. Mineralisation of PHCs can thus be directly correlated to CO_2 production. Estimation of metabolic activity through O_2 consumption detects only the activity of aerobic and facultative microorganisms (Stotzky 1997).

Static respiratory systems, in which soils are isolated in chambers, are low cost, simple and inexpensive to use, and suitable for short-term laboratory studies (Wollum 1994). Sampling of the head space of the chamber does not disturb the sample, and soil moisture is not lost by continuous air flow or alkali solutions (Wollum 1994). Aeration is unnecessary if CO_2 concentrations in the headspace do not rise above 2% during incubation, a condition avoided by using large jars as chambers (Wollum 1994).

Soil samples of 50 g dw were moistened to field capacity (-33 kPa water tension) to maximise microbial activity and eliminate experimental error from varying water contents in the samples (Stotzky 1997). While a moisture content of 50% water-holding capacity is commonly used for respiration testing (Bhattacharyya et al. 2003; Emmerling et al. 2000; Kästner et al. 1994; Lindstrom et al. 1999; Ericksson et al. 2000; Margesin et al. 2000a; Margesin et al. 2000b; Bundy et al. 2002; Maliszewska-Kordybach and Smreczak 2003), the volumetric water content of the sieved samples was above this at 0.15. Field capacity and available water holding capacity were estimated at 0.2 and 0.1 cm^3 water cm^{-3} soil, respectively, based upon soil texture of 73% sand and 13% clay. Field capacity thus represents 50% of the saturation estimate of 0.4 cm^3 water cm^{-3} soil (Saxton and Nelson 2000). Available water holding capacity was expected to be higher in samples amended with compost. The desired water content of the samples was therefore 0.2 on a volumetric basis. These samples were allowed to equilibrate in plastic bags at room temperature for approximately 40 hours (Stotzky 1997).

Moistened soil samples were placed into 2 L Mason jars with sealable lids and butyl rubber septa (Lindstrom et al. 1999). Three empty jars were maintained as blanks. Jars were sealed and incubated at room temperature in darkness. Carbon dioxide in the headspace of each jar were sampled with a gas-tight syringe and analysed by gas chromatography (GC). The GC used was a Varian Chrompack Micro-GC CP-2003, equipped with a 10 m Molsieve 5Å column and thermal conductivity detector for O_2 analysis and a 10 m Poraplot Q column and flame ionization detector for CO_2 analysis, with CP Maitre Elite Version 3.1 software. The concentration of CO_2 measured from the blanks was subtracted from the GC readings to calculate amount of CO_2 produced by the samples. Carbon dioxide and O_2 concentrations were measured after two and five days of incubation. Aerobic respiration was reported as μg C (g oven-dry soil)$^{-1}$ hour^{-1}.

2.3.6 Statistical Analyses

Data from the 10^{-6} and 10^{-5} soil dilutions were analysed for plate counts of aerobic heterotrophic microorganisms and viable fungi, respectively. Colony morphological richness and diversity index were counted on 10^{-5} soil dilution PCA plates. These dilutions were the best representations of colony types and occurrences in the series.

Of the tested parameters, four had sample sizes large enough for parametric analyses: aerobic heterotrophic and viable fungal population sizes, colony morphological richness and Shannon-Weaver diversity for colony morphology. These plate count data had a sample size of 16 per contamination x amendment x sampling depth treatment; sample size per treatment was four for all other variables, and the assumption made that parametric tests could not be used for such a low sample size (Zar 1999). The Shapiro-Wilk calculation for normality available on SPSS 13.0 has high power (Zar 1999). The Shapiro-Wilk coefficient, skewness and kurtosis coefficients, histograms, Q-Q plots and box plots indicated more than half the data for plate count parameters were not normally distributed. Non-parametric tests were thus used for all parameters, with a probability *P* value of 0.05 to determine significance.

Boxplots created by SPSS 13.0 identified extreme cases as values further than three box lengths from either box edge, where the box is the interquartile range (range of values between 25th and 75th percentiles). Outliers were identified as values between 1.5 and three box lengths from either box edge. Normality of distribution plate count data were tested after removal of extreme cases and outliers identified by the SPSS 13.0 program.

The non-parametric Wilcoxon signed-rank t-test was used to determine if values from the 0 to 10 and 10 to 20 cm depths differed significantly (Zar 1999). The Wilcoxon signed-rank test is used to compare two related samples. The Wilcoxon signed-rank t-test was used to determine that aerobic respiration rates measured on days two and five of incubation were not significantly different; the mean aerobic respiration rate from these measurements was then calculated and used as a parameter.

The Scheirer-Ray-Hare test was applied as a non-parametric equivalent of a two-factor ANOVA (Dytham 2003). This test was performed to determine if parameters differed significantly between contamination, amendment and contamination x amendment treatments. Each data set was ranked, then an ANOVA performed on the ranked data. The total corrected sum of squares for each test was the combined total sum of squares for the contamination, amendment, contamination x amendment interaction and error factors. The total mean square was calculated by dividing the total corrected sum of squares by the degrees of freedom (Scheirer et al. 1976). The sums of squares for the three factors listed above were then divided by the total mean square to calculate test statistics for each factor. The probability values were calculated by subtracting the cumulative probability that a value from the chi-squared distribution would be less than the test statistic, with the degrees of freedom of the factor (function CDF.CHISQ). Levene's equality of variance test was run at the same time to estimate homogeneity of variances in each factor (Dytham 2003).

If the Scheirer-Ray-Hare test indicated that only contamination or amendment was a significant factor, or the applicable post hoc test indicated significant difference between amendment treatments, the Kruskal Wallis test was conducted as a non-parametric one-factor ANOVA on the parameter for that factor only (Dytham 2003). This test has higher power than the Scheirer-Ray-Hare test to determine if there is a significant difference between treatments for that factor. The Mann-Whitney U test, the equivalent to Wilcoxon signed ranks test and the Kruskal Wallis test for two independent samples, was used when the differing factor was contamination and therefore only two groups were tested (Dytham 2003).

Because the contamination factor had only two groups (no contamination and crude oil contamination), post hoc tests could not be applied. Tukey's honestly significant difference (HSD) test and Tamhane's T2 test were run with the two-factor ANOVA as post hoc tests to determine which amendment treatments significantly differed from each other (Dytham 2003). Tukey's HSD range test identifies homogenous subsets of means and assumes that variances are equal, while the conservative Tamhane's test runs multiple pairwise comparisons based upon the t-test and does not assume equal variances. Levene's equality of variance test was thus used to determine which post hoc test applied.

The non-parametric Spearman rank-order test was used to calculate correlation, and the parametric Pearson product-moment correlation test was run. The standard Pearson test coefficient used to the correlation is r, and r^2 yields the amount of variation in the dependent variable y explained by variation in the independent variable x. The Spearman correlation coefficient, r_s, also indicates strength and direction of the association between x and y, but should not be compared to r (Dytham 2003).

2.3.6.1 Analysis of soil dilution effect

Examination of the Petri dishes showed that the number of colonies per plate, for both bacterial colonies on PCA and fungi on Martin's media, did not decrease logarithmically with level of dilution but in a linear fashion. The decrease in plate counts (y) over the soil dilution level (x) appeared linear from soil dilutions 10^3 to 10^7 for PCA, with an increase at 10^8. The decrease for Martin's media over soil dilutions 10^2 to 10^5 also appeared linear (data not shown). The slopes of each soil dilution series per treatment were tested, averaged, and the means compared with Microsoft Excel ANOVA two-factor test without replication. The slopes for PCA counts and Martin's media plate count slopes did not differ significantly between contamination treatment, amendment treatment or sampling depth. The soil dilution effect was thus identical between treatments for both PCA and Martin's media counts. To determine if the soil dilution effect was the same for aerobic heterotroph counts on PCA as for fungal counts on Martin's media, for each contamination x amendment treatment a value was calculated by dividing the slope for Martin's media counts by the slope for PCA counts. These values were compared using ANOVA and no significant difference was found for either contamination or amendment treatments. The difference in slope between aerobic heterotrophic counts and fungal counts, then, is the same between treatments, and a correction for the soil dilution effect on PCA counts could also be used on Martin's media counts.

2.4 RESULTS AND DISCUSSION

2.4.1 Weather

Temperatures were similar to climate normals for the area over the study period. Rainfall was generally below normal in both growing seasons with the exceptions of August 2003 and July 2004, when above average rainfall occurred (Table A3).

2.4.2 Soil Dilution Effect

Counts per Petri plate for both PCA and Martin's media did not decrease logarithmically with logarithmic increase in soil dilution (counts on plates inoculated at 10^5 soil dilution were not ten times less than those at 10^4 soil dilution) (Figure 2.2). This was initially attributed to inadequate dissolution of soil aggregates during preparation of the initial dilution, resulting in decreased aggregation and increased number of microorganisms as the suspension was further diluted. Soil dilution suspensions were prepared by shaking each bottle by hand 12 times, which later was theorised to be insufficient to break up soil clumps. This error will be referred to as the 'soil dilution effect'.

If the soil dilution effect was caused by repeated shaking of the dilution suspensions, then the effect should have been smaller or non-existent for soil dilutions shaken 24 to 72 times instead of 12. The same dilution effect was observed as before, with no apparent difference between suspensions prepared from initial soil dilutions shaken different times (Figure 2.3). The soil dilution effect did not result from inadequate dilution of the initial soil solutions. Since many colonies were large and often covered whole plates, a possible cause of the dilution effect could have been steric hindrance of colony development.

2.4.3 Soil and Vegetation Properties and PHC Degradation

Results from Graham's (2005) MSc research were used in this thesis to characterise conditions under which microbial populations developed and functioned and are presented in Tables 2.2 to 2.5 and Figure 2.4.

2.4.3.1 Vegetation and soil physical and chemical properties

Oxygen concentrations measured in situ at the end of the field trial did not differ from ambient air concentrations, varying between 19 and 22% in each contamination and amendment treatment at each soil depth.

The nonparametric Wilcoxon paired t-test could not detect differences with sampling depth for soil chemical properties (Tables 2.2 and 2.3), but leaching effects were visible in the data. There were few to no significant differences between compost and compost + fertiliser treatments.

Crude oil contamination had no effect on soil properties, with the following exceptions. In fertilised and non-amended soils C:N ratios were significantly higher with contamination. Compost amendment had significant effects on all soil properties except C:N ratios for non-contaminated soils and total leachate (Tables 2.2 and 2.3). Although concentrations of Cd, Cu, Pb and Zn increased with compost application (Table 2.1), compost benefits compensated for any vegetation or microbial toxicity from metal loading (Graham 2005).

Vegetation cover and root biomass in non-contaminated soils was highest for compost treatments and lowest for fertiliser treatments (Table 2.2). For contaminated soils, vegetation cover and root biomass were highest for compost + fertiliser and next highest for compost only treatments; no significant difference occurred between fertiliser and control treatments as vegetation cover and root biomass were near zero in soils not amended with compost. Crude oil did not significantly lower vegetation cover and root biomass in compost-amended soils, with the exception of root biomass for the compost treatment. Compost + fertiliser increased vegetation cover of contaminated soil over that in non-contaminated soil, while compost only and fertiliser only amendments failed to compensate for crude oil toxicity.

2.4.3.2 PHC degradation

Compost amendment significantly increased loss of TEH from soil in all CCME fractions (Table 2.4; Figure 2.4). No significant difference between compost treatments, or between fertiliser and control treatments, was detected. Leachate volumes did not differ significantly between amendment treatments, as discussed previously, indicating TEH loss from contaminated soils could not be wholly attributed to leaching. The amount of PHCs lost differed with CCME fraction, being highest for fraction 2 and lowest for fraction 4, demonstrating the recalcitrance of fractions 3 and 4. Significant reduction in fraction 4 was observed only in compost-amended treatments (Table 2.5). Loss of fraction 2 from crude oil was 82%, 97% and 79% in control, compost and fertiliser treatments, respectively. Fraction 3 decreased 32%, 81% and 40% in control, compost and fertiliser treatments. Fraction 4 from contaminated soils decreased 13%, 70% and 26% for control, compost and fertiliser treatments. Total losses for fractions 2, 3 and 4 in contaminated soils were 2229, 1684 and 75 mg kg^{-1} in non-amended soils; 1325, 3517 and 1915 mg kg^{-1} in compost amended soils; and 1640, 1748 and 411 mg kg^{-1} in fertiliser only treatments.

Compost applications added 830 and 1183 mg kg^{-1} of fraction 3 and 4 PHCs, respectively, to non-contaminated soils. At the end of the field trial values were 908 and 504 mg kg^{-1} (Table 2.4). Ten of 16 replicates showed an increase of 250 mg kg^{-1} in fraction 3, with decreases of 214 mg kg^{-1} in the remaining replicates. All replicates had lowered concentrations of fraction 4, however, with an average decrease of 679 mg kg^{-1}.

2.4.4 Microbiological Parameters

2.4.4.1 Differences between sampling depth

Significant differences with sampling depth were observed in colony morphological richness, Shannon-Weaver colony morphological diversity indices and aerobic heterotrophic plate counts (Tables 2.7 to 2.9). The affected colony morphological richness values were from contaminated soil amended with compost and/or fertiliser, and for non-contaminated fertiliser and control treatments. Significant differences in aerobic heterotrophic plate counts were detected in fertiliser amendment treatments with no crude oil contamination. With extreme cases and outliers removed from the data set, differences in aerobic heterotrophic and fungal plate counts between sampling depths were also significant in contaminated soils amended with fertiliser only. Only the non-contaminated fertiliser amendment did not show a significant difference with sampling depth for colony morphological diversity.

There was no observable pattern in differences between sampling depths for aerobic heterotrophs and colony morphological diversity indices; half the values measured at 0 to 10 cm were higher than those from 10 to 20 cm, and half were lower. The only consistent patterns, which could not be proven statistically, were that aerobic heterotrophic plate counts in soils amended with compost + fertiliser were higher in the 10 to 20 cm depth and colony morphological diversity in fertiliser-amended soils was higher in the soil surface. Differences between sampling depth averaged 0.77 x 10^7 colony forming units (CFU) g^{-1} soil for aerobic heterotrophic microorganisms in compost + fertiliser treatments, and 0.2 for Shannon-Weaver diversity indices in fertiliser only treatments.

Colony morphological richness was higher in the 0 to 10 cm depth than in the 10 to 20 cm depth, with the exception of contaminated soils amended with compost + fertiliser and the non-contaminated, non-amended control. The differences detected were small, ranging from 0.4 to 2.6, and not likely relevant considering the high variability inherent in the richness measurements (Figure 2.6).

2.4.4.2 Aerobic heterotrophic microoganisms

The high variability and non-normal distribution of data from plate counts for aerobic heterotrophic microorganisms yielded little information upon statistical analysis. This may be due in part to the limitations of culturing techniques, which detect less than 10% of total soil microorganisms (Zuberer 1994; Trevors 1998; Alexander 1999). Other studies also failed to find a crude oil contamination effect on total soil bacteria populations (Song and Bartha 1990; Nichols et al. 1997; Lindstrom et al. 1999). Although no significant difference between contamination treatments was found, aerobic heterotrophic bacteria appeared higher with crude oil contamination for the fertiliser only treatment but lower for compost treatments (Table 2.6). Populations in compost and fertiliser only treatments were 1.73 and 2.14 x 10^7 CFU g^{-1} soil, respectively, in contaminated soils, compared to 2.37 and 1.58 x 10^7 CFU g^{-1} soil, respectively, for non-contaminated soils.

Compost amendment had a small but significant positive effect on aerobic heterotrophic microorganism population size in control soils, with an increase of 0.95 from the control treatment of 1.37 x 10^7 CFU g^{-1} soil, while fertiliser increased aerobic heterotrophs by 0.96 x 10^7 CFU g^{-1} soil at 0 to 10 cm only. No significant differences between amendments were detected in contaminated soils (Table 2.7) (Figure 2.5).

2.4.4.3 Viable fungi

Fungal populations in non-contaminated soils were increased by compost amendment; this effect was lessened for the compost + fertiliser treatment, but was still significant (Figure 2.6). Populations in control soils were 11.40 and 6.90 x 10^5 CFU g^{-1} soil, respectively, for the compost only and compost + fertiliser treatment, compared to 4.40 x 10^5 CFU g^{-1} soil in the non-amended treatment. Fertiliser only amendment significantly decreased fungi below those in non-amended soils for both contamination treatments, with counts in non-contaminated soil dropping to 2.78 x 10^5 CFU g^{-1} soil, and fungal populations in compost only treatments significantly higher than those in the compost + fertiliser treatments at $P < 0.10$ in both contamination treatments.

Viable fungi were reduced by crude oil contamination, although the drop was not significant in non-amended control treatments (Table 2.7). Fungal populations in contaminated soil were 6.33, 2.97 and 0.93 x 10^5 CFU g^{-1} soil for compost only, compost + fertiliser, and fertiliser only treatments, and 3.20 x 10^5 CFU g^{-1} soil in the non-amended control. Fungal populations in non-amended soil may have been so low that toxicity from crude oil could not significantly affect them, whereas larger populations in compost-amended treatments exhibited a more detectable drop with contamination. Fungal populations in compost-amended soils were therefore not statistically different from those in non-amended soils in contaminated treatments.

2.4.4.4 Colony morphological richness estimated at 10^5 dilution in aerobic heterotrophic PCA plates

Colony morphological richness increased with crude oil contamination. This was significant in fertiliser-amended soils at both depths with an increase of 3.7, and for the compost + fertiliser treatment at 10 to 20 cm at 2.1. At $P < 0.10$, differences were also significant at 0 to 10 cm for compost only and for no amendment treatments at 1.9 and 1.2, respectively (Table 2.7).

In control soils the number of colony types per Petri dish at 0 to 10 cm was 5.6, 7.3, 6.5 and 6.5 for control, compost only, fertiliser only, and compost + fertiliser treatments, respectively. At 10 to 20 cm these averages were 7.1, 6.9, 3.9 and 5.9. Contaminated soils with no amendment, compost only, fertiliser only and compost + fertiliser yielded richness values of 7.8, 9.2, 9.5 and 6.3 at 0 to 10 cm, and 7.3, 6.3, 7.4 and 8.0, respectively, at 10 to 20 cm. Fertiliser amendment had a small, significant, negative effect on colony morphological richness in non-contaminated soil at 10 to 20 cm, which was not observed at other depths and contamination treatments. In fact fertiliser application appeared to increase richness in contaminated soils, although no significant difference was observed. Little significant difference was found among compost treatments, and between compost treatments and non-amended controls, for non-contaminated and contaminated soils. No difference in colony morphological richness among amendment treatments was detected in crude oil contaminated soils (Figure 2.7).

2.4.4.5 Shannon-Weaver diversity index for colony morphology

Colony morphological diversity significantly increased in soils treated with crude oil for all four amendment treatments, with increases at 0 to 10 cm of 0.3, 0.7, 0.0 and 0.5 for compost only, fertiliser only, compost + fertiliser, and no amendment treatments, respectively (Table 2.7). At 10 to 20 cm this effect was smaller with respective increases of 0.1, 0.4, 0.3 and 0.2 (Figure 2.8). These results agree with those of Bundy et al. (2002), where diesel fuel contamination caused microbial community composition to diverge greatly from those in control soils without subsequently converging after 103 days. Lors et al. (2004) also reported PHC contamination increased richness, and thus diversity, of bacterial populations. Saul et al. (2005) found that weathered hydraulic and lubrating oil contamination decreased Shannon-Weaver diversity indices, calculated from clone libraries constructed from restriction fragment length polymorphism analysis (RFLP). Diversity indices in control and contaminated soils were 3.70 and 2.93, a highly significant ($P < 0.001$) result attributed to domination by hydrocarbon-degrading microorganisms in contaminated soils. These values are representative for Antarctic and temperate soils (Saul et al. 2005), demonstrating that colony morphology evaluation underestimated the genetic diversity of the microbial communities.

Diversity indices in control soils at 0 to 10 cm were 1.1, 1.2, 0.9 and 1.5 for no amendment, compost only, fertiliser only, and compost + fertiliser treatments; these values were 1.2, 1.3, 0.9 and 1.3, respectively, at 10 to 20 cm. Results suggested that fertiliser application slightly decreased colony morphological diversity, particularly in non-contaminated soil. The highest diversity values in both contamination treatments were in the compost treatments, although neither statistically significant nor biologically relevant from those in non-amended soils. Diversity indices in compost + fertiliser treatments were significantly higher than those in control treatments for non-contaminated soils, however, but not in contaminated soils.

2.4.4.6 Aromatic and aliphatic PHC degrading microorganisms

The INT assay appeared to work well on plates treated with the PAH mixture, although poor results with this technique on PAHs have been reported (Wrenn and Venosa 1996). No significant increase in positive scores was observed after addition of an electron donor solution designed to increase the sensitivity of the INT assay.

The population of microorganisms capable of degrading aliphatic PHCs was greater than that of the aromatic PHC degrading population in both contamination treatments, as determined by the Wilcoxon paired t-test. No significant effect of crude oil on aromatic PHC degrading MPNs or aliphatic PHC degrading MPNs was detected (Table 2.7), but trends of higher PHC degrading microbial population sizes in contaminated soil treatments compared to control treatments could be seen in boxplots. Changes in population size for aromatic PHC degrading microorganisms with crude oil contamination ranged from -1.74 x 10^5 to 3.56 x 10^5 MPN g^{-1} soil, and from -8.66 x 10^5 to 6.27 x 10^5 MPN g^{-1} soil for aliphatic PHC degrading microorganisms (Figure 2.9).

Aliphatic PHC degrading microorganisms were significantly increased by compost amendment, but increases were not significant for aromatic PHC degrader MPNs (Table 2.7). The compost + fertiliser treatment significantly increased aromatic PHC degrading microorganisms above those in controls in non-contaminated soils only, but the difference was significant in both contamination treatments for aliphatic PHC degrading microorganisms. Counts in the compost + fertiliser treatment appeared higher than those in the compost only treatment for aliphatic PHC degrading microorganisms, although the effect was significant only in non-contaminated soils at combined depths ($P < 0.05$) and 10 to 20 cm ($P < 0.10$). Aromatic PHC degrading microorganisms were 0.54 and 0.91 x 10^5 MPN g^{-1} soil in the compost only and compost + fertiliser treatments in control soils, compared to 0.25 x 10^5 MPN g^{-1} soil in the non-amended control. In soils contaminated with crude oil the non-amended treatment exhibited 1.02 x 10^5 MPN g^{-1} soil aromatic PHC degrading microorganisms, while values in the compost only and compost + fertiliser treatments were 1.24 and 0.68 x 10^5 MPN g^{-1} soil, respectively. Aliphatic PHC degrading microorganisms were 1.45 and 2.61 x 10^5 MPN g^{-1} soil in the compost only and compost + fertiliser treatments in control soils, compared to 0.60 x 10^5 MPN g^{-1} soil in the non-amended control. In contaminated soils aliphatic PHC degrading microorganisms were 1.19, 2.13 and 3.67 x 10^5 MPN g^{-1} soil in the control, compost only and compost + fertiliser treatments, respectively.

Fertiliser appeared to decrease aromatic and aliphatic PHC degrading microorganisms in both contamination treatments, but these differences were not statistically significant (Table 2.7). Aromatic PHC degrading microorganisms numbered 0.10 and 0.34 x 10^5 MPN g^{-1} soil in control and contaminated soils, respectively, while the aliphatic PHC degrading population was 0.31 and 0.36 x 10^4 MPN g^{-1} soil in control and contaminated soils, respectively. Aromatic and aliphatic PHC degrading microbial populations in compost-amended treatments were significantly higher than those amended with fertiliser in both contamination treatments, however.

Aliphatic PHC degrading microorganisms significantly increased with colony morphological diversity, microbial biomass and C mineralisation rates. Increases in aliphatic PHC degrading microorganisms, microbial biomass C, CO_2 production and vegetation cover were in turn correlated with PHC reduction, illustrating that PHCs were consumed by soil microorganisms. The sharp increase in fungal populations in soils amended with compost agrees with the results of Kästner and Mahro (1996), in which compost application increased populations of actinomycetes and fungi in PAH-contaminated soil by three orders of magnitude. The lack of response of total aerobic heterotrophs, as well as PHC degrading microorganisms, is common in PHC biodegradation experiments (Salanitro 2001). Reasons why PHC decreases in soil may not correspond to microbial enumeration assays include low culturability of soil microorganisms, sequestration of PHCs in soil, and microbial metabolism of PHCs to CO_2 without cell growth (Salanitro 2001).

2.4.4.7 Microbial biomass carbon

Crude oil contamination decreased microbial biomass C, but not significantly (Table 2.7). The change in microbial biomass carbon with contamination was 4.08, 15.52, 0.90 and 3.48 mg C g^{-1} soil for control, compost, fertiliser and compost + fertiliser treatments.

Microbial biomass C increased with compost amendment, with no significant differences between the two compost amendment treatments. Microbial biomass was 35.81 and 21.68 mg C g^{-1} soil in compost only and compost + fertiliser treatments in control soils and 20.29 and 18.19 mg C g^{-1} soil in contaminated soils, respectively, compared to 9.06 and 2.98 mg C g^{-1} soil with no amendment in control and contaminated soils. No significant difference in microbial biomass C was detected between fertiliser and control treatments in contaminated soils, but fertiliser application significantly decreased microbial biomass C in control soils to 3.38 mg C g^{-1} soil and not significantly to 4.29 mg C g^{-1} soil in contaminated soils (Figure 2.10).

2.4.4.8 Aerobic respiration rate

Aerobic respiration rates measured on days two and five of incubation did not differ significantly, except for the control treatment; the respiration rate was slightly higher on day two than on day five for non-amended soils. The difference was not considered biologically significant and was attributed to outliers in the data (Table A6). The mean rate between the two was therefore used in correlations (Figure 2.11).

Crude oil contamination had no significant effect on aerobic respiration rates (Table 2.8). Compost amendment increased aerobic respiration rates above those in control and fertiliser treatments. No significant difference between compost amended treatments was detected, although results for the compost only treatment were higher than those of the compost + fertiliser treatment in non-contaminated soils. In non-contaminated soils, the fertiliser treatment decreased aerobic respiration rate at significance level $P < 0.10$ ($P < 0.05$ for combined depths). Aerobic respiration rates in non-contaminated, non-amended soils were 0.43 μg C g^{-1} soil $hour^{-1}$, compared to 1.51 and 0.28 μg C g^{-1} soil $hour^{-1}$ in compost treatments and in fertiliser only treatments. In contaminated soils respiration rates were 0.64, 1.40 and 0.49 μg C g^{-1} soil $hour^{-1}$ for control, compost and fertiliser treatments, respectively. These results agree with those from the field trial of Emmerling et al. (2000), in which 50, 250 and 500 Mg ha^{-1} dw mature compost were applied to sandy coal mine soils in Germany and soil microbial properties measured over two years. Compost application significantly increased soil respiration, phosphatase activity and invertase activity, with greater effects at higher application rates.

2.4.4.9 Aerobic respiratory ratio

Aerobic respiratory ratios in contaminated, non-amended soil were >1 on day two of incubation, indicating that more CO_2 was being produced than O_2 consumed. This may have been an error of calculation and instrumentation, in which too little O_2 was consumed to be accurately measured. These ratios dropped to equal those in non-contaminated soils by day five of incubation. The fertiliser treatment also exhibited an elevated respiratory ratio in contaminated soils compared to non-contaminated soils on day two of incubation, but this difference was significant for combined depths only (Table 2.9). Respiratory ratios measured on day five were therefore used for correlations.

Aerobic respiratory ratios significantly increased with compost amendment in comparison to fertiliser and non-amended treatments on both days and both contamination treatments (Figure 2.12). Data from day five of incubation indicated that fertiliser amendment significantly decreased aerobic respiratory ratio in both contamination treatments. Respiratory ratios were 0.5, 0.3 and 0.7 for no amendment, fertiliser, and combined compost treatments, respectively, in both contamination treatments.

Oxygen consumption will equal CO_2 production, and respiratory ratios thus equal unity, only when hydrocarbons are fully mineralised. Petroleum hydrocarbons may not be fully mineralised, however, yielding stable smaller hydrocarbons but not CO_2. Respiratory ratios (moles CO_2 produced/moles O_2 consumed) are thus lower when PHC degradation is incomplete. Brook et al. (2001) calculated degradation rates for diesel fuel in contaminated soils incubated and aerated in sealed respirometers at constant temperature and O_2 concentration for 60 days at C:N ratios of 40:1 and 20:1. Degradation rate constants calculated by CO_2 production and by O_2 consumption were up to 30 and 60 times lower, respectively, than those calculated from TPH loss. These results were attributed to incomplete mineralisation of TPHs, after errors from denitrification and CO_2 sorption by soil carbonates were considered and calculated to be insignificant. The increase in respiratory ratios in compost-amended soils may thus indicate more efficient PHC degradation, as well as increased mineralisation of soil organic C due to its higher content in compost treatments, compared to non-amended controls.

2.4.4.10 Nitrification and PHC degradation

Nitrate concentrations in soil amended with $(NH_4)_2SO_4$ fertiliser increased over the field trial, demonstrating that nitrifying soil microorganisms were oxidising the large NH_4^+ supply to nitrite (NO_2^-) and then NO_3^-. Nitrification may be responsible for the reduced respiratory ratios calculated in fertiliser-amended soils, consuming O_2 without a corresponding production of CO_2. Although pHs below 4.5 inhibit autotrophic and (to a lesser degree) heterotrophic nitrifying microorganisms (Myrold 1999), the acidity in fertiliser-amended soils was likely not sufficient to limit nitrification where NH_4^+ was highly available.

Salminen et al. (2004) observed high CO_2 production without mineral oil degradation during aerobic incubation of heavily contaminated soils. Neither methane nor volatile hydrocarbons were detected in the headspace of the respirometers. Neither CO_2 or CH_4 production or mineral oil loss was observed in sterilised controls under either anaerobic or aerobic incubation. This was attributed to breakdown of organic matter. Though most of the easily degradable organic matter added to soils from compost should have been consumed by the end of the 14 month field trial, this may have contributed to the elevated CO_2 respiration rates in compost-amended soils.

Lindstrom et al. (1999) reported no net difference in NH_4^+ concentrations between soils with and without crude oil contamination, but nitrification was absent from contaminated samples. Ammonia monooxygenase (AMO) catalyses oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH) for autotrophic nitrifying bacteria. This enzyme has been shown to oxidize low molecular weight straight-chain alkanes, alkenes (Hyman et al. 1988), naphthalene and small, water-soluble PAHs (Chang et al. 2002). The presence of PHCs thus inhibits nitrification by competitive interaction with AMO (Hyman et al. 1988; Chang et al. 2002) and through the toxicity of the resulting metabolites on AMO (Chang et al. 2002). Acetylene and other alkynes also inhibit the enzyme (Chang et al. 2002). Rates of hydrocarbon oxidation by AMO are dependent upon NH_4^+ ion concentrations (Hyman et al. 1988), and both NH_3 and hydrocarbon oxidation increase with increasing NH_4^+ concentrations (Chang et al. 2002). Humic acid application increases growth and nitrifying activity of autotrophic nitrifying bacteria, stimulating both NH_4^+ oxidation and NO_2^- depletion, but is not used as a C or nutrient source (Vallini et al. 1997). Inhibition of nitrification by crude oil thus explains the lower NO_3^- observed in contaminated soils compared to control soils (Table 2.3).

2.4.5 Correlations

Of the parameters tested, only four had sample sizes large enough for non-parametric correlations to be calculated per contamination and amendment treatment: aerobic heterotrophic population size, viable fungal population size, colony morphological richness and Shannon-Weaver diversity for colony morphology. Positive correlation between fungal and aerobic heterotrophic population counts was significant only for compost only treatments. Colony morphological richness and diversity were significantly related in all treatments, with Spearman correlation coefficients r_s of 0.69 for 0 to 10 cm and 0.81 for 10 to 20 cm (Tables 2.10).

Correlations were not run on soil properties with PHC degradation or microbiological parameters because compost had such a large effect that the data would be separated into two groups, indicating a significant effect where there may be none. For example, compost amendment increased microbial biomass C, PHC degradation and vegetation cover, but also increased SAR and metal content; a correlation performed on SAR and metals data would indicate that increases in SAR and heavy metal concentrations yield significant increases in microbial metabolism and plant productivity, whereas it is more probable that these properties were all influenced by compost amendment and the relationship between them is thus an error of analysis. For remaining microbiological parameters and PHC data, correlations were calculated for all treatments combined; correlation values below are for combined depths unless otherwise specified.

Total extractable hydrocarbon loss from contaminated soils was significantly and positively correlated with the following parameters at the following r_s values: aliphatic PHC degrading microorganisms at 0.42, microbial biomass C at 0.57, mean respiration rate at 0.76, respiratory ratio measured on day five of incubation at 0.74, vegetation cover at 0.75 and root biomass at 0.76 (Table 2.11; Figure 2.13).

Fraction 2 PHC loss from contaminated soils was significantly and positively correlated with the following parameters at the following r_s values: aliphatic PHC degrading microorganisms at 0.51, microbial biomass C at 0.62, mean respiration rate at 0.76, respiratory ratio measured on day five of incubation at 0.74, vegetation cover at 0.76 and root biomass at 0.72.

Fraction 3 PHC loss from contaminated soils was positively and significantly correlated with aliphatic PHC degrading microorganisms at $r_s = 0.42$, microbial biomass C at $r_s = 0.58$, mean respiration rate at $r_s = 0.79$, respiratory ratio measured on day five of incubation at $r_s = 0.77$, vegetation cover at $r_s = 0.77$ and root biomass at $r_s = 0.79$.

Fraction 4 PHC loss from contaminated soils was significantly and positively correlated with microbial biomass C at $r_s = 0.49$, mean respiration rate at $r_s = 0.59$, respiratory ratio measured on day five of incubation at $r_s = 0.61$, vegetation cover at $r_s = 0.73$ and root biomass at $r_s = 0.74$.

Vegetation cover and root biomass were both significantly correlated with the following parameters at the following respective r_s values: fungi population size at 0.36 and 0.28, aliphatic PHC degrading microbial population at 0.31 and 0.30 (correlation with root biomass was not significant under parametric Pearson test), microbial biomass C at 0.60 and 0.63, and mean respiration rate at 0.46 and 0.60, respectively (Table 2.12). Root biomass was also significantly and positively correlated with respiratory ratio measured on day five of incubation at r_s 0.65 (Table 2.12).

Fungi population estimates and microbial biomass C were significantly correlated for a r_s of 0.48. Aromatic and aliphatic PHC degrading microbial populations were strongly and positively correlated at $r_s = 0.64$, although this correlation was not significant with the parametric Pearson test. Shannon-Weaver diversity indices for colony morphology were positively correlated with the following parameters for 10 to 20 cm only: aromatic PHC degrading microbial population at $r_s = 0.46$, aliphatic PHC degrading microbial population at $r_s = 0.50$ and microbial biomass C at $r_s = 0.40$ (Tables 2.13 and 2.14).

Microbial biomass C and MPNs of aromatic PHC degrading microorganisms were significantly correlated with $r_s = 0.27$ (not significant under parametric Pearson test), and correlation with aliphatic PHC degrading microorganisms yielded positive significant correlation of r_s 0.43. Microbial biomass C was significantly correlated with mean respiration rate at $r_s = 0.64$, and correlation with respiratory ratio measured on day five of incubation yielded a significant and positive relationship of $r_s = 0.68$.

Fungal counts were significantly correlated with mean respiration rate and with respiratory ratio measured on day five of incubation at r_s values of 0.39 and 0.34, respectively.

Aliphatic PHC degrading microorganisms and mean respiration rate were significantly correlated with $r_s = 0.33$, and correlation with respiratory ratio measured on day five of incubation yielded and r_s of 0.40. Aromatic PHC degrading microorganisms were significantly and positively correlated with respiratory ratio measured on day five of incubation at $r_s = 0.35$, but correlation with mean respiration rate was not significant.

2.5 CONCLUSIONS

- Crude oil contamination decreased viable fungi, microbial biomass C and vegetation invasion, but slightly increased colony morphological richness and diversity. Increases were also observed for PHC-degrading microorganisms, but these differences were not statistically significant.
- Compost amendment increased aerobic heterotrophic microorganisms, viable fungi, microbial biomass C and aerobic respiration, but had no detectable effect on colony morphological richness or diversity.
- Inorganic fertiliser amendment increased aerobic heterotrophic microorganisms and colony morphological richness but decreased viable fungi, and had no detectable effect upon microbial biomass C or aerobic respiration.
- PHC degradation was positively correlated with root biomass, aliphatic PHC degrading microbial populations, microbial biomass C and aerobic respiration rates and ratios. Compost amendment increased PHC degradation in all fractions in contaminated soils, especially fractions 3 and 4.
- No biologically significant differences in soil chemical or microbiological properties were found between 0 to 10 cm depth and 10 to 20 cm depth.

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Table 2.1 Initial compost and soil treatment characterisation by Graham (2005)

	Compost	Soil	Soil + fertiliser	Soil + compost	Soil + compost + fertiliser
Sand (%)		73 ± 0			
Silt (%)		14 ± 1			
Clay (%)		13 ± 0			
Bulk density (Mg m^{-3})	0.53 ± 0.01	1.88 ± 0.02	1.88 ± 0.02	1.74 ± 0.01	1.74 ± 0.01
Total extractable hydrocarbons (mg kg^{-1})	9033 ± 491	76 ± 15	55 ± 13	2053 ± 292	2058 ± 163
Total organic carbon (%)	19.9 ± 0.6	1.8 ± 0.1	1.6 ± 0.2	6.4 ± 0.3	6.3 ± 0.1
Total Kjeldhal nitrogen (%)	1.85 ± 0.01	0.13 ± 0.01	0.20 ± 0.01	0.52 ± 0.02	0.54 ± 0.01
Carbon:nitrogen ratio	11.2 ± 0.4	14.5 ± 0.5	8.6 ± 0.7	13.0 ± 0.2	12.1 ± 0.3
Ammonium (mg kg^{-1})	7.5 ± 0.2	6.1 ± 0.3	811.8 ± 0.2	10.7 ± 1.5	638.2 ± 22.9
Nitrate (mg kg^{-1})	1867 ± 75.4	8.5 ± 0.4	8.3 ± 0.5	344.4 ± 19.9	317.5 ± 28.8
pH	7.7 ± 0.0	6.6 ± 0.0	6.6 ± 0.0	7.1 ± 0.0	7.2 ± 0.0
Electrical conductivity (dS m^{-1})	16.73 ± 0.47	0.49 ± 0.02	0.51 ± 0.02	10.18 ± 0.28	9.89 ± 0.11
Sodium adsorption ratio	15.9 ± 0.4	0.3 ± 0.0	0.3 ± 0.0	9.7 ± 0.2	9.5 ± 0.1
Sodium (mg L^{-1})	2387 ± 89	11 ± 0	11 ± 0	1399 ± 40	1355 ± 22
Cadmium (mg kg^{-1})	3.0 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	1.0 ± 0.1	1.0 ± 0.1
Copper (mg kg^{-1})	200 ± 3	13 ± 1	11 ± 1	63 ± 6	63 ± 3
Lead (mg kg^{-1})	152 ± 3	11 ± 1	10 ± 0	47 ± 4	46 ± 3
Zinc (mg kg^{-1})	646 ± 10	54 ± 3	48 ± 2	205 ± 18	212 ± 13

Numbers in brackets indicate standard error of the mean
Taken directly from Graham 2005

Table 2.2 Soil physical properties and vegetation at end of experiment from Graham (2005)

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Vegetation cover, %	N/A	56.2 ± 6.2 ^{bA}	73.8 ± 6.5 ^{aA}	5.4 ± 3.3 ^{cA}	5.4 ± 3.3 ^{cA}	55.6 ± 11.4^{abA}	76.9 ± 8.7 ^{aA}	32.5 ± 11.3^{bB}	62.5 ± 0.0 ^{aB}
Root biomass, Mg m^{-3}	N/A	42.20 ± 12.16^{bA}	92.90 ± 21.24^{aA}	5.06 ± 1.77^{cA}	6.53 ± 3.90^{cA}	142.61 ± 54.59^{aA}	104.57 ± 30.20^{aA}	4.82 ± 2.24^{cA}	75.10 ± 23.81^{aB}
Final bulk density, Mg m^{-3}	N/A	1.10 ± 0.02^{aA}	1.09 ± 0.04^{aA}	1.27 ± 0.01^{bA}	1.28 ± 0.02^{bA}	1.23 ± 0.03^{bA}	1.08 ± 0.03^{aA}	1.30 ± 0.02^{bA}	1.00 ± 0.22^{abA}
Initial soil moisture	N/A	16.2 ± 1.0	17.8 ± 1.1	7.6 ± 0.4	7.2 ± 0.4	12.0 ± 0.0	No data	6.8 ± 0.0	8.6 ± 0.6
Final soil moisture, % mass basis	0 to 10	23.4 ± 9.8 ^{aA}	13.4 ± 0.4 ^{aA}	11.8 ± 2.4 ^{aA}	15.2 ± 1.5 ^{aA}	23.6 ± 4.9 ^{aA}	32.3 ± 14.2^{aA}	12.1 ± 0.6 ^{bA}	12.1 ± 0.4 ^{bA}
	10 to 20	16.1 ± 3.4 ^{aA}	13.8 ± 1.1 ^{aA}	13.8 ± 0.5 ^{aA}	13.2 ± 0.8 ^{aA}	30.9 ± 14.1^{aA}	15.4 ± 0.9 ^{aA}	11.6 ± 0.4 ^{bA}	11.5 ± 0.2 ^{bA}
	Combined	19.8 ± 5.0 ^{aA}	13.6 ± 0.5 ^{aA} *	12.8 ± 1.2 ^{aA} *	14.2 ± 0.9 ^{aA} *	27.2 ± 7.1 ^{aA}	23.8 ± 7.3 ^{aA}	11.8 ± 0.3 ^{bA} *	11.8 ± 0.3 ^{bA}
Initial TOC	N/A	7.4 ± 0.6	7.2 ± 0.5	3.0 ± 0.3	3.5 ± 0.4	6.0 ± 0.6	6.3 ± 0.2	1.5 ± 0.1	2.0 ± 0.2
Final total organic carbon (TOC), %	0 to 10	6.1 ± 0.2 ^{aA}	6.4 ± 0.6 ^{aA}	2.2 ± 0.2 ^{bA}	2.8 ± 0.3 ^{bA}	6.4 ± 0.6 ^{aA}	6.1 ± 0.4 ^{aA}	2.3 ± 0.1 ^{bA}	2.5 ± 0.4 ^{bA}
	10 to 20	6.0 ± 0.3 ^{aA}	5.6 ± 0.4 ^{aA}	2.4 ± 0.1 ^{bA}	2.8 ± 0.2 ^{bA}	6.9 ± 0.8 ^{aA}	6.4 ± 0.4 ^{aA}	2.0 ± 0.2 ^{bA}	1.9 ± 0.3 ^{bA}
	Combined	6.0 ± 0.2 ^{aA} *	6.0 ± 0.4 ^{aA} *	2.2 ± 0.1 ^{bA} *	2.8 ± 0.2 ^{cA} *	6.7 ± 0.5 ^{aA}	6.2 ± 0.3 ^{aA}	2.2 ± 0.1 ^{bA} *	2.2 ± 0.3 ^{bA}

Table 2.2 continued

	Sampling depth, cm	Crude oil contamination C	Crude oil contamination CF	Crude oil contamination F	Crude oil contamination NA	No contamination C	No contamination CF	No contamination F	No contamination NA
Initial base saturation, %	N/A	46.2 ± 1.9	47.0 ± 3.7	33.4 ± 1.7	32.7 ± 0.5	63.7 ± 8.6	60.0 ± 1.1	32.3 ± 0.6	33.4 ± 0.7
Final base saturation, %	0 to 10	47.0 ± 2.2 aA	50.5 ± 1.4 aA	34.8 ± 1.5 bA	33.5 ± 1.0 bA	49.0 ± 2.8 aA	50.5 ± 1.0 aA	29.5 ± 1.0 bA	35.2 ± 2.5 bA
	10 to 20	46.2 ± 2.1 aA	50.5 ± 0.6 aA	35.0 ± 0.9 bA	36.2 ± 1.9 bA	51.5 ± 3.7 aA	49.0 ± 0.8 aA	31.5 ± 1.4 bA	32.8 ± 1.4 bA
	Combined	46.6 ± 1.4 aA	50.5 ± 0.7 aA	34.9 ± 0.8 bA	34.9 ± 1.1 bA	50.2 ± 2.2 aA	49.8 ± 0.7 aA *	30.5 ± 0.9 bA	34.0 ± 1.4 bA
2003 leachate production, mL	N/A	1530 ± 490 ^{aA}	2554 ± 792 ^{aA}	1820 ± 370 ^{aA}	1852 ± 505 ^{aA}	1234 ± 503 ^{aA}	372 ± 233 aA	405 ± 235 aA	2 ± 2 ^{aB}
Total leachate production, mL	N/A	4807 ± 773 ^{aA}	6237 ± 1494 ^{aA}	7495 ± 598 ^{aA}	8268 ± 485 ^{aA}	4772 ± 738 ^{aA}	2798 ± 394 ^{aA}	5070 ± 609 ^{aA}	2907 ± 267 ^{aB}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

* Sampling depth means are significantly different at $P < 0.05$, when extreme cases and outliers are removed

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Upper case letters indicate contamination treatment means per amendment treatment are significantly different across that depth at $P < 0.05$

Taken from Graham 2005

Table 2.3 Soil chemical properties from Graham (2005)

	Sampling depth, cm	Crude oil contamination C	Crude oil contamination CF	Crude oil contamination F	Crude oil contamination NA	No contamination C	No contamination CF	No contamination F	No contamination NA
Initial soil pH	N/A	7.2 ± 0.1	7.3 ± 0.1	6.9 ± 0.0	6.9 ± 0.0	7.2 ± 0.0	7.3 ± 0.0	6.6 ± 0.0	6.6 ± 0.0
Final soil pH	0 to 10	7.6 ± 0.0^{aA}	7.2 ± 0.0^{bA}	5.1 ± 0.0^{cA}	7.2 ± 0.0^{bA}	7.8 ± 0.0^{aA}	7.4 ± 0.0^{bA}	5.2 ± 0.1^{cA}	7.2 ± 0.0^{bA}
	10 to 20	7.6 ± 0.0^{aA}	7.3 ± 0.0^{bA}	5.3 ± 0.1^{cA}	7.2 ± 0.0^{bA}	7.7 ± 0.0^{aA}	7.2 ± 0.1^{bA}	5.0 ± 0.1^{cA}	7.2 ± 0.0^{bA}
	Combined	7.6 ± 0.0^{aA} *	7.2 ± 0.0^{bA}	5.2 ± 0.0^{cA} *	7.2 ± 0.0^{bA} *	7.7 ± 0.0^{aA} *	7.3 ± 0.0^{bA}	5.1 ± 0.1^{cA} *	7.2 ± 0.0^{dA} *
Initial soil EC	N/A	10.03 ± 0.22	10.56 ± 0.55	0.68 ± 0.17	0.51 ± 0.02	10.01 ± 0.42	9.78 ± 0.25	0.48 ± 0.00	0.45 ± 0.03
Final soil electrical conductivity (EC), dS m^{-1}	0 to 10	2.90 ± 0.41 ^{aA}	3.90 ± 0.22 ^{aA}	3.68 ± 0.21 ^{aA}	0.62 ± 0.16 ^{bA}	3.28 ± 0.21 ^{aA}	4.22 ± 0.22 ^{aA}	3.62 ± 0.18 ^{aA}	0.50 ± 0.04 ^{bA}
	10 to 20	5.40 ± 0.71 ^{abA}	5.18 ± 0.21 ^{aA}	0.68 ± 0.17 ^{bA}	0.45 ± 0.03 ^{cA}	5.50 ± 0.56 ^{aA}	9.40 ± 1.90 ^{aA}	5.10 ± 0.62 ^{aA}	0.58 ± 0.02 ^{bA}
	Combined	4.15 ± 0.61 ^{aA} *	4.54 ± 0.28 ^{aA} *	4.04 ± 0.17 ^{aA} *	0.54 ± 0.08 ^{bA}	4.39 ± 0.50 ^{aA} *	6.81 ± 1.32 ^{aA}	4.36 ± 0.41 ^{aA} *	0.54 ± 0.03 ^{bA} *
Initial SAR	N/A	8.6 ± 0.2	9.2 ± 0.4	0.3 ± 0.0	0.3 ± 0.0	9.6 ± 0.7	9.7 ± 0.2	0.3 ± 0.0	0.3 ± 0.0
Final sodium adsorption ratio (SAR)	0 to 10	4.5 ± 0.7^{aA}	2.2 ± 0.3^{bA}	0.2 ± 0.0^{cA}	0.7 ± 0.1^{dA}	5.0 ± 0.2^{aA}	2.6 ± 0.5^{bA}	0.2 ± 0.1^{cA}	0.4 ± 0.1^{cA}
	10 to 20	7.4 ± 0.7^{aA}	5.2 ± 0.6^{aA}	0.2 ± 0.0^{bA}	0.6 ± 0.1^{cA}	8.5 ± 0.7^{aA}	7.0 ± 0.7^{aA}	0.1 ± 0.0^{bA}	0.4 ± 0.1^{cA}
	Combined	6.0 ± 0.7^{aA} *	3.7 ± 0.6^{bA} *	0.2 ± 0.0^{cA} *	0.7 ± 0.1^{dA} *	6.8 ± 0.7^{aA} *	4.8 ± 0.9^{aA} *	0.1 ± 0.0^{cA} *	0.4 ± 0.1^{dA}
Initial TKN	N/A	0.60 ± 0.03	0.63 ± 0.04	0.27 ± 0.01	0.20 ± 0.03	0.49 ± 0.06	0.56 ± 0.02	0.21 ± 0.02	0.14 ± 0.02

Table 2.3 continued

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Final total Kjeldhal nitrogen (TKN), %	0 to 10	0.45 ± 0.00^{aA}	0.42 ± 0.02^{aA}	0.15 ± 0.01^{bA}	0.15 ± 0.02^{bA}	0.50 ± 0.04^{aA}	0.48 ± 0.03^{aA}	0.19 ± 0.01^{bA}	0.18 ± 0.04^{bA}
	10 to 20	0.45 ± 0.03^{aA}	0.41 ± 0.03^{aA}	0.180 ± 0.02^{bA}	0.16 ± 0.02^{bA}	0.54 ± 0.05^{aA}	0.50 ± 0.02^{aA}	0.18 ± 0.01^{bA}	0.15 ± 0.03^{bA}
	Combined	0.45 ± 0.01^{aA} *	0.42 ± 0.02^{aA} *	0.16 ± 0.01^{bA} *	0.15 ± 0.01^{bA} *	0.52 ± 0.03^{aA}	0.49 ± 0.02^{aA} *	0.18 ± 0.01^{bA}	0.16 ± 0.02^{bA}
Initial NH_4^+	N/A	6.3 ± 0.4	606.2 ± 0.2	809.0 ± 0.1	3.4 ± 0.1	12.3 ± 3.1	615.7 ± 1.6	811.9 ± 0.5	6.4 ± 0.5
Final ammonium (NH_4^+), mg kg^{-1}	0 to 10	3.4 ± 0.2^{aA}	4.8 ± 0.4^{abA}	46.2 ± 18.0^{bA}	2.4 ± 0.2^{cA}	4.6 ± 0.3^{abA}	5.8 ± 0.6^{aA}	73.4 ± 32.4^{abA}	3.1 ± 0.5^{bcA}
	10 to 20	3.6 ± 0.5^{acA}	5.5 ± 0.7^{abA}	90.4 ± 34.4^{bA}	2.4 ± 0.2^{cA}	4.4 ± 0.6^{abA}	6.4 ± 0.5^{aA}	71.2 ± 34.9^{abA}	2.8 ± 0.2^{bcA}
	Combined	3.5 ± 0.2^{bA} *	5.2 ± 0.4^{cA} *	68.3 ± 20.2^{dA} *	2.4 ± 0.1^{aA} *	4.5 ± 0.3^{bA} *	6.1 ± 0.4^{cA} *	72.3 ± 22.0^{abcdA} *	3.0 ± 0.3^{aA} *
Initial C:N	N/A	12.7 ± 0.5	11.7 ± 0.2	11.1 ± 0.5	17.8 ± 0.4	12.7 ± 0.3	11.8 ± 0.7	7.6 ± 0.6	15.3 ± 0.9
Final carbon to nitrogen (C:N) ratio	0 to 10	13.6 ± 0.5^{aA}	15.4 ± 1.8^{abA}	14.3 ± 0.5^{aA}	19.1 ± 1.0^{bA}	12.8 ± 0.3^{aA}	12.9 ± 0.8^{aA}	12.0 ± 0.8^{aB}	15.3 ± 1.4^{aA}
	10 to 20	13.3 ± 0.7^{aA}	13.4 ± 0.5^{aA}	13.2 ± 0.6^{aA}	18.5 ± 1.2^{bA}	12.8 ± 0.3^{aA}	12.8 ± 0.7^{aA}	11.4 ± 1.1^{aA}	13.3 ± 0.8^{aB}
	Combined	13.4 ± 0.4^{aA}	14.4 ± 0.9^{aA} *	13.7 ± 0.4^{aA} *	18.8 ± 0.7^{cA}	12.8 ± 0.2^{abA}	12.8 ± 0.5^{abA}	11.7 ± 0.6^{bB} *	14.3 ± 0.8^{aB} *

Table 2.3 continued

	Sampling depth, cm	Crude oil contamination C	CF	F	NA	No contamination C	CF	F	NA
Initial NO_3^-	N/A	338.8 ± 12.8	287.2 ± 10.2	3.6 ± 0.4	13.4 ± 7.4	309.8 ± 51.2	360.8 ± 11.4	7.6 ± 0.8	8.2 ± 0.8
Final nitrate (NO_3^-), mg kg^{-1}	0 to 10	2.3 ± 0.2^{aA}	5.8 ± 3.8^{aA}	65.6 ± 5.0^{bA}	1.5 ± 0.3^{aA}	4.0 ± 0.5^{aB}	15.2 ± 8.6^{abA}	46.8 ± 8.6^{bA}	3.4 ± 0.5^{aB}
	10 to 20	2.0 ± 0.3^{aA}	3.4 ± 1.2^{aA}	93.9 ± 12.2^{bA}	1.4 ± 0.2^{aA}	2.3 ± 0.1^{aA}	241.9 ± 59.4^{bB}	116.3 ± 31.2^{bA}	2.9 ± 0.4^{aB}
	Combined	2.2 ± 0.2^{aA} *	4.6 ± 1.9^{aA} *	79.7 ± 8.1^{bA} *	1.5 ± 0.2^{cA} *	3.2 ± 0.4^{aA} *	128.6 ± 51.1^{bB} *	81.5 ± 19.9^{bA} *	3.2 ± 0.3^{aB} *
Final sulphate (SO_4^{-2}), mg kg^{-1}	0 to 10	94 ± 30^{aA}	594 ± 86^{bA}	424 ± 27^{bA}	23 ± 12^{aA}	179 ± 23^{aA}	616 ± 193^{abA}	372 ± 38^{bA}	10 ± 1^{cA}
	10 to 20	347 ± 37^{aA}	1120 ± 35^{bA}	926 ± 40^{bA}	13 ± 1^{cA}	254 ± 82^{aA}	1115 ± 49^{bA}	1090 ± 7^{bA}	13 ± 1^{cA}
	Combined	221 ± 53^{aA}	857 ± 108^{bA}	675 ± 97^{bA}	18 ± 6^{cA}	217 ± 42^{aA}	865 ± 132^{bA}	731 ± 137^{bA}	12 ± 1^{cA}
Initial Ca^{+2}	N/A	1082.5 ± 27.8	1095.0 ± 37.8	129.3 ± 38.9	91.7 ± 3.2	1125.0 ± 19.4	1100.0 ± 30.0	91.70 ± 1.6	84.8 ± 5.3
Final calcium (Ca^{+2}), mg kg^{-1}	0 to 10	302.0 ± 31.7^{aA}	715.2 ± 21.8^{bA}	814.0 ± 65.3^{bA}	93.8 ± 29.5^{cA}	277.5 ± 39.4^{aA}	646.0 ± 50.2^{bA}	750.2 ± 24.6^{bA}	75.5 ± 7.2^{cA}
	10 to 20	572.0 ± 66.9^{aA}	743.5 ± 11.6^{bA}	855.2 ± 52.1^{bA}	65.8 ± 5.8^{cA}	453.0 ± 55.0^{aA}	1139.0 ± 290.6^{abA}	957.0 ± 115.0^{bA}	91.8 ± 4.0^{cA}
	Combined	437.0 ± 61.5^{aA} *	729.4 ± 12.6^{bA} *	834.6 ± 39.4^{bA} *	79.8 ± 14.9^{cA}	365.2 ± 45.6^{aA} *	892.5 ± 165.3^{bA}	853.6 ± 67.0^{bA} *	83.6 ± 4.9^{cA}

Table 2.3 continued

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Initial K^+	N/A	465.2 ± 23.4	569.5 ± 72.8	4.1 ± 0.7	4.8 ± 0.7	608.0 ± 101.9	572.0 ± 21.4	4.9 ± 0.9	7.0 ± 0.9
Final potassium (K^+), mg kg^{-1}	0 to 10	141.2 ± 20.0^{aA}	170.5 ± 34.1^{aA}	10.5 ± 0.6 ^{bA}	4.5 ± 0.6^{cA}	170.2 ± 12.0^{aA}	233.5 ± 39.9^{aA}	12.5 ± 1.8 ^{bA}	6.0 ± 0.9^{cA}
	10 to 20	199.8 ± 12.6^{aA}	173.0 ± 12.4^{aA}	16.8 ± 1.3 ^{bA}	3.5 ± 0.6^{cA}	278.2 ± 73.7^{aA}	263.5 ± 62.8^{aA}	18.5 ± 2.6 ^{bA}	3.8 ± 0.2^{cA}
	Combined	170.5 ± 15.6^{aA} *	171.8 ± 16.8^{aA} *	13.6 ± 1.4 ^{bA} *	4.0 ± 0.5^{cA}	224.2 ± 40.1^{aA} *	248.5 ± 34.9^{aA} *	15.5 ± 1.9 ^{bA} *	4.9 ± 0.6^{cA} *
Initial Mg^{+2}	N/A	242.8 ± 4.6	257.8 ± 12.4	19.5 ± 5.5	14.52 ± 0.5	267.2 ± 7.9	265.5 ± 9.4	14.28 ± 0.3	14.00 ± 0.8
Final magnesium (Mg^{+2}), mg kg^{-1}	0 to 10	62.8 ± 7.4 ^{aA}	117.5 ± 13.1^{bA}	138.0 ± 4.0 ^{bA}	22.2 ± 3.1 ^{cA}	60.5 ± 8.5 ^{aA}	115.0 ± 12.2^{abA}	128.8 ± 15.3^{bA}	17.50 ± 2.6 ^{cA}
	10 to 20	126.8 ± 10.1^{aA}	145.5 ± 3.4 ^{aA}	183.5 ± 6.9 ^{bA}	16.2 ± 1.6 ^{cA}	107.5 ± 14.1^{aA}	230.8 ± 60.6^{abA}	214.5 ± 35.9^{bA}	19.50 ± 2.3 ^{cA}
	Combined	94.8 ± 13.4 ^{aA} *	131.5 ± 8.2 ^{bA} *	160.8 ± 9.4 ^{bA} *	19.2 ± 2.0 ^{cA}	84.0 ± 11.7 ^{aA} *	172.9 ± 36.0^{bA}	171.6 ± 24.2^{bA} *	18.50 ± 1.7 ^{cA} *
Initial Na^+	N/A	1208 ± 27	1312 ± 83	14 ± 2	11 ± 1	1372 ± 101	1378 ± 55	11 ± 1	10.75 ± 0.250
Final sodium (Na^+), mg kg^{-1}	0 to 10	335 ± 58^{aA}	243 ± 31^{aA}	24 ± 4^{bA}	28 ± 3^{bA}	350 ± 27^{aA}	279 ± 55^{aA}	15.00 ± 8.879^{bA}	14.25 ± 2.955^{bA}
	10 to 20	761 ± 105 ^{aA}	592 ± 68^{aA}	24 ± 4^{bA}	22 ± 6^{bA}	780 ± 114 ^{aA}	1006 ± 227 ^{aA}	16.25 ± 1.548^{bA}	15.50 ± 3.329^{bA}
	Combined	548 ± 98^{aA} *	418 ± 74^{aA} *	24 ± 3^{bA} *	25 ± 3^{bA} *	565 ± 98^{aA} *	643 ± 175 ^{aA} *	15.63 ± 4.179^{bA}	14.88 ± 2.074^{bA}

Table 2.3 continued

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Initial Na^+	N/A	1208 ± 27	1312 ± 83	14 ± 2	11 ± 1	1372 ± 101	1378 ± 55	11 ± 1	10.75 ± 0.250
Final sodium (Na^+), mg kg^{-1}	0 to 10	335 ± 58^{aA}	243 ± 31^{aA}	24 ± 4^{bA}	28 ± 3^{bA}	350 ± 27^{aA}	279 ± 55^{aA}	15.00 ± 8.879^{bA}	14.25 ± 2.955^{bA}
	10 to 20	761 ± 105^{aA}	592 ± 68^{aA}	24 ± 4^{bA}	22 ± 6^{bA}	780 ± 114^{aA}	1006 ± 227^{aA}	16.25 ± 1.548^{bA}	15.50 ± 3.329^{bA}
	Combined	548 ± 98^{aA} *	418 ± 74^{aA} *	24 ± 3^{bA} *	25 ± 3^{bA} *	565 ± 98^{aA} *	643 ± 175^{aA} *	15.63 ± 4.179^{bA}	14.88 ± 2.074^{bA}
Initial Mn^{+2}	N/A	712 ± 56	705 ± 56	895 ± 59	998 ± 113	748 ± 91	702 ± 48	942 ± 108	942 ± 117
Final manganese (Mn^{+2}), mg kg^{-1}	0 to 10	678 ± 55^{aA}	825 ± 147^{aA}	862 ± 103^{aA}	815 ± 59^{aA}	1162 ± 465^{aA}	738 ± 40^{aA}	938 ± 160^{aA}	820 ± 54^{aA}
	10 to 20	678 ± 93^{aA}	675 ± 51^{aA}	680 ± 39^{aA}	1040 ± 168^{aA}	682 ± 55^{aA}	828 ± 139^{aA}	905 ± 118^{aA}	932 ± 188^{aA}
	Combined	678 ± 50^{aA}	750 ± 77^{aA}	771 ± 61^{abA}	928 ± 93^{bA}	922 ± 235^{bA}	782 ± 69^{bA}	921 ± 92^{bA}	876 ± 93^{bA}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

* Sampling depth means are significantly different at $P < 0.05$, when extreme cases and outliers are removed

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Upper case letters indicate contamination treatment means per amendment treatment are significantly different across that depth at $P < 0.05$

Taken from Graham 2005

Table 2.4 Total decreases in petroleum hydrocarbon contamination over experiment from Graham (2005)

Parameter	Sampling depth, cm	C	CF	F	NA
Crude oil contamination treatments					
Initial TEH concentration	N/A	9025 ± 287	10300 ± 1470	8275 ± 211	10075 ± 622
Final total extractable	0 to 10	3150 ± 487 ^{a}	2825 ± 502 ^{a}	4450 ± 479 ^{a}	6275 ± 703 ^{b}
hydrocarbon (TEH)	10 to 20	2950 ± 290 ^{a}	2450 ± 189 ^{a}	4300 ± 649 ^{a}	5450 ± 703 ^{a}
concentration	Combined	3050 ± 265 ^{a}	2638 ± 258 ^{a}	4375 ± 374 ^{b}	5862 ± 486 ^{b}
Initial fraction 2 PHCs	N/A	1396 ± 57	1339 ± 179	2069 ± 35	2730 ± 194
Final fraction 2 (>C_{10} to C_{16})	0 to 10	41 ± 6 ^{a}	36 ± 6 ^{ab}	424 ± 68 ^{ab}	530 ± 89 ^{b}
petroleum hydrocarbons	10 to 20	51 ± 10 ^{a}	40 ± 2 ^{ab}	433 ± 80 ^{ab}	473 ± 54 ^{b}
(PHC)	Combined	46 ± 6 ^{a}	38 ± 3 ^{ab}	429 ± 49 ^{b}	501 ± 49 ^{c}
Initial fraction 3 PHCs	N/A	5015 ± 130	5492 ± 781	4425 ± 121	5278 ± 322
Final fraction 3 (>C_{16} to C_{34})	0 to 10	1889 ± 240 ^{a}	1730 ± 278 ^{a}	2809 ± 319 ^{ab}	3920 ± 476 ^{b}
PHC	10 to 20	1788 ± 140 ^{a}	1539 ± 118 ^{ab}	2544 ± 343 ^{b}	3266 ± 338 ^{b}
	Combined	1838 ± 130 ^{a}	1634 ± 145 ^{a}	2677 ± 222 ^{b}	3593 ± 297 ^{b}
Initial fraction 4 PHCs	N/A	2662 ± 183	3324 ± 484	1690 ± 64	1845 ± 63
Final fraction 4 (>C_{34}) PHC	0 to 10	1228 ± 253 ^{ab}	1066 ± 201 ^{b}	1248 ± 161 ^{a}	1826 ± 225 ^{a}
	10 to 20	1116 ± 168 ^{ab}	902 ± 84 ^{b}	1310 ± 273 ^{a}	1712 ± 348 ^{a}
	Combined	1171 ± 142 ^{a}	984 ± 106 ^{a}	1279 ± 147 ^{b}	1770 ± 193 ^{b}
No contamination					
Initial fraction 3 PHCs	N/A	728 ± 61	931 ± 77	40 ± 9	63 ± 13
Final fraction 3 PHC	0 to 10	957 ± 69 ^{a}	972 ± 105 ^{a}	30 ± 16 ^{a}	27 ± 10 ^{a}
	10 to 20	844 ± 54 ^{a}	850 ± 105 ^{a}	19 ± 10 ^{a}	38 ± 12 ^{a}
	Combined	900 ± 46 ^{a}	911 ± 72 ^{a}	24 ± 9 ^{a}	33 ± 7 ^{a}

Table 2.4 continued

Parameter	Sampling depth, cm	C	CF	F	NA
No contamination					
Initial fraction 4 PHCs	N/A	1037 ± 115	1329 ± 94	10 ± 2	34 ± 8
Final fraction 4 PHC	0 to 10	516 ± 76 ^{a}	530 ± 83 ^{a}	7 ± 2 ^{b}	5 ± 0 ^{b}
	10 to 20	476 ± 65 ^{a}	494 ± 76 ^{a}	6 ± 2 ^{b}	6 ± 2 ^{b}
	Combined	496 ± 47 ^{a}	512 ± 52 ^{a}	7 ± 1 ^{b}	6 ± 1 ^{c}

Table 2.5 Loss of petroleum hydrocarbons as fraction of total extractable hydrocarbon content at start of experiment

Parameter	Sampling depth, cm	C	CF	F	NA
% Loss of total extractable hydrocarbons (TEH) added from crude oil	0 to 10	77 ± 7^{a}	79 ± 10^{a}	46 ± 6^{ab}	37 ± 4^{b}
	10 to 20	78 ± 5^{ab}	83 ± 5^{a}	48 ± 7^{bc}	46 ± 4^{c}
	Combined	78 ± 4^{a}	81 ± 5^{a}	47 ± 4^{b}	42 ± 3^{b}
% Loss of fraction 2 ($C_{>10\text{-}16}$) petroleum hydrocarbons (PHCs) from crude oil	0 to 10	97 ± 0^{a}	98 ± 1^{a}	80 ± 4^{b}	80 ± 3^{b}
	10 to 20	97 ± 1^{a}	97 ± 1^{a}	79 ± 4^{b}	83 ± 1^{b}
	Combined	97 ± 0^{a}	97 ± 0^{a}	79 ± 3^{b}	82 ± 2^{b}
% Loss of fraction 3 ($C_{>16\text{-}34}$) PHCs added from crude oil	0 to 10	79 ± 5^{a}	79 ± 10^{a}	36 ± 8^{b}	23 ± 5^{b}
	10 to 20	78 ± 4^{a}	82 ± 6^{a}	43 ± 7^{b}	38 ± 2^{b}
	Combined	78 ± 3^{a}	81 ± 5^{a}	39 ± 5^{b}	32 ± 4^{b}
% Loss of fraction 4 ($C_{>34}$) PHCs added from crude oil	0 to 10	56 ± 17^{a}	63 ± 15^{a}	27 ± 7^{a}	8 ± 8^{a}
	10 to 20	59 ± 13^{ab}	70 ± 9^{b}	26 ± 12^{a}	18 ± 7^{a}
	Combined	58 ± 10^{a}	66 ± 8^{a}	26 ± 7^{b}	13 ± 5^{b}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

Sampling depth means were not significantly different at $P < 0.05$

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Table 2.6 Change in microbiological parameters in crude oil contaminated treatments in comparison to non-contaminated, non-amended control treatment

	Sampling depth, cm	No amendment		Fertiliser		Compost		Compost + fertiliser	
		Total	Relative	Total	Relative	Total	Relative	Total	Relative
Aerobic heterotrophics, 10^7 colony forming units (CFU) g^{-1}	Combined	0.76	+56%	1.69	+123%	0.81	+59%	1.18	+86%
Viable fungi, 10^5 CFU g^{-1}	Combined	-0.59	-15%	-2.36	-59%	1.75	44%	-1.11	-28%
Aromatic petroleum hydrocarbon (PHC) degrading microorganisms, 10^5 MPN g^{-1}	Combined	0.66	+183%	-0.02	-6%	0.88	+246%	0.32	+89%
Aliphatic PHC degraders, 10^5 MPN g^{-1}	Combined	0.59	+98%	-0.24	-41%	1.53	+256%	3.07	+513%
Microbial biomass carbon (C), mg C g^{-1}	Combined	-4.08	-45%	-4.77	-53%	11.2	+124%	9.13	+101%
Colony morphological richness at 10^{-5} soil dilution	0 to 10	2.2	+39%	3.9	+69%	3.6	+63%	0.8	+13%
	10 to 20	0.2	+3%	0.6	+8%	-0.4	-6%	0.9	+12%
Shannon-Weaver index for colony morphological diversity	0 to 10	0.4	+32%	0.5	+50%	0.4	+39%	0.3	+26%
	10 to 20	0.1	+10%	0.0	0%	0.2	+14%	0.2	+19%
Vegetation cover, %	N/A	-57.12	-91%	-57.12	-91%	-6.25	-10%	11.25	+18%
Root biomass, Mg m^{-3}	N/A	-68.6	-91%	-70.0	-93%	-32.9	-44%	17.8	+24%
Mean aerobic respiration rate, μg C g^{-1} soil $hour^{-1}$	Combined	0.18	+39%	0.03	+7%	0.94	+204%	0.94	+203%
Respiratory ratio, moles CO_2/moles O_2	Combined	0.05	+10%	-0.11	-22%	0.20	+41%	0.20	+42%

Underlining indicates that total difference between value and value for non-contaminated, non-amended control treatment is significant at $P < 0.05$

Table 2.7 Soil microbiological properties

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Aerobic heterotrophs, 10^7 colony forming units (CFU) g^{-1} soil	0 to 10	1.83 ± 0.38^{aA}	2.05 ± 0.68^{aA}	2.88 ± 0.48^{aA}	2.14 ± 0.61^{aA}	3.02 ± 0.69^{bA}	1.73 ± 0.21^{abA}	1.99 ± 0.16^{aA}	1.03 ± 0.15^{cA}
	10 to 20	2.52 ± 0.74^{aA}	3.05 ± 1.07^{aA}	3.24 ± 1.91^{aA}	2.12 ± 0.45^{aA}	2.48 ± 0.47^{abA}	3.11 ± 0.42^{bA}	0.98 ± 0.21^{aA}	1.71 ± 0.46^{aA}
	Combined	2.18 ± 0.41^{aA}	2.55 ± 0.63^{aA}	3.06 ± 0.97^{aA} *	2.13 ± 0.37^{aA}	2.75 ± 0.41^{aA}			1.37 ± 0.25^{cA}
Viable fungi, 10^5 CFU g^{-1} soil	0 to 10	6.06 ± 1.31^{aA}	2.95 ± 0.56^{aA}	0.75 ± 0.17^{bA}	4.58 ± 1.02^{aA}	15.00 ± 2.92^{bB}	7.80 ± 1.14^{bB}	2.05 ± 0.39^{aB}	3.04 ± 0.59^{aA}
	10 to 20	5.41 ± 1.41^{aA}	2.80 ± 0.40^{aA}	2.51 ± 2.24^{bA}	2.20 ± 0.43^{aA}	9.77 ± 2.77^{aA}	6.00 ± 0.85^{aB}	2.95 ± 0.61^{bB}	4.92 ± 1.07^{abA}
	Combined	5.74 ± 0.95^{aA}	2.88 ± 0.34^{aA}	1.63 ± 1.12^{bA} *	3.39 ± 0.58^{aA}	12.39 ± 2.04^{bB}	6.90 ± 0.72^{bB}	2.50 ± 0.36^{aB}	3.98 ± 0.62^{aA}
Colony morphological richness per plate at 10^5 soil dilution	0 to 10	9.2 ± 0.5 ^{aA}	6.4 ± 0.6 ^{bA}	9.5 ± 0.5 ^{aA}	7.8 ± 1.0 ^{abA}	7.3 ± 0.7 ^{abA}	6.5 ± 0.6 ^{aA}	6.1 ± 0.7 ^{abB}	5.6 ± 0.5 ^{bA}
	10 to 20	6.7 ± 0.6 ^{aA}	8.0 ± 0.8 ^{aA}	7.7 ± 0.4 ^{aA}	7.3 ± 1.0 ^{aA}	6.9 ± 0.7 ^{aA}	5.9 ± 0.5^{aB}	3.9 ± 0.4 ^{bB}	7.1 ± 0.5 ^{aA}
	Combined				7.6 ± 0.7^{bA}	7.1 ± 0.5^{aA}	6.2 ± 0.4^{aA}		
Microbial biomass carbon, mg C g^{-1} soil	0 to 10	25.66 ± 3.81^{aA}	14.18 ± 4.95^{abA}	5.34 ± 2.95^{bA}	5.76 ± 1.05^{bA}	29.92 ± 4.47^{aA}	26.84 ± 4.32^{aA}	4.48 ± 1.12^{bA}	9.944 ± 1.124^{cA}
	10 to 20	14.93 ± 1.84^{aA}	22.20 ± 1.48^{aA}	3.24 ± 1.01^{bA}	4.12 ± 0.79^{bA}	41.71 ± 19.38^{aA}	16.51 ± 3.07^{acA}	2.29 ± 0.64^{bA}	8.17 ± 1.05^{cA}
	Combined	20.29 ± 2.82^{aA}	18.19 ± 2.83^{aA}	4.29 ± 1.50^{bA}	2.98 ± 0.68^{bA}	35.81 ± 9.47^{aA}	21.68 ± 3.14^{aA}	3.38 ± 0.76^{bA}	9.06 ± 0.79^{cA}

Table 2.7 continued

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Shannon-Weaver diversity index for colony morphology	0 to 10	1.5 ± 0.1^{aA}	1.4 ± 0.1^{aA}	1.6 ± 0.1^{aA}	1.4 ± 0.2^{aA}	1.2 ± 0.1^{aB}	1.4 ± 0.6^{bA}	0.9 ± 0.1^{aB}	1.1 ± 0.1^{aB}
	10 to 20	1.4 ± 0.1^{abA}	1.5 ± 0.1^{aA}	1.2 ± 0.1^{bA}	1.4 ± 0.2^{aA}	1.3 ± 0.1^{aA}	1.2 ± 0.09^{aB}	0.8 ± 0.1^{bB}	1.2 ± 0.1^{aA}
	Combined							0.9 ± 0.1^{bB}	
Aromatic petroleum hydrocarbon (PHC) degraders, 10^5 MPN g^{-1} soil	0 to 10	1.40 ± 0.80^{aA}	0.42 ± 0.32^{aA}	0.10 ± 0.02^{aA}	1.74 ± 0.91^{aA}	0.25 ± 0.13^{aA}	0.80 ± 0.32^{aA}	0.17 ± 0.06^{aA}	0.44 ± 0.24^{aA}
	10 to 20	1.09 ± 0.46^{aA}	0.94 ± 0.57^{aA}	0.57 ± 0.51^{aA}	0.29 ± 0.10^{aA}	0.83 ± 0.49^{abA}	1.03 ± 3.34^{aA}	0.04 ± 0.02^{bA}	0.28 ± 0.12^{abA}
	Combined	1.24 ± 0.43^{aA}	0.68 ± 0.32^{abA}	0.34 ± 0.25^{bA}	1.02 ± 0.50^{abA}	0.54 ± 0.26^{abA}	0.91 ± 0.22^{aA}	0.10 ± 0.04^{bA}	0.36 ± 0.13^{abA}
Aliphatic PHC degraders, 10^5 Most Probable Number (MPN) g^{-1} soil	0 to 10	1.74 ± 0.79^{aA}	3.81 ± 1.31^{aA}	0.24 ± 0.12^{aA}	1.88 ± 1.13^{aA}	1.84 ± 1.27^{aA}	2.89 ± 1.95^{aA}	0.52 ± 0.36^{aA}	0.37 ± 0.14^{aA}
	10 to 20	2.53 ± 1.26^{aA}	3.54 ± 1.15^{aA}	0.47 ± 0.44^{abA}	0.49 ± 0.08^{bA}	1.07 ± 0.51^{abA}	2.33 ± 0.17^{aA}	0.12 ± 0.05^{bA}	0.83 ± 0.59^{abA}
	Combined	2.13 ± 0.71^{abA}	3.67 ± 0.81^{bA}	0.355 ± 0.215^{cA}	1.19 ± 0.59^{acA}	1.45 ± 0.65^{acA}	2.61 ± 0.91^{bA}	0.31 ± 0.19^{cA}	0.60 ± 0.29^{cA}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

* Sampling depth means are significantly different at $P < 0.05$, when extreme cases and outliers are removed

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Upper case letters indicate contamination treatment means per amendment treatment are significantly different across that depth at $P < 0.05$

Table 2.8 Aerobic respiration rates measured in soil samples incubated without aeration

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Respiration rate on day two, μg^{-1} C g^{-1} soil hr^{-1}	0 to 10	1.49 ± 0.06^{aA}	1.50 ± 0.19^{aA}	0.83 ± 0.39^{abA}	0.58 ± 0.02^{bA}	2.03 ± 0.32^{cA}	1.41 ± 0.06^{aA}	0.27 ± 0.01^{abcA}	0.54 ± 0.05^{bA}
	10 to 20	1.37 ± 0.19^{abA}	1.37 ± 0.150^{bA}	0.37 ± 0.14^{cA}	0.79 ± 0.11^{acA}	1.41 ± 0.20^{acA}	1.11 ± 0.22^{aA}	0.16 ± 0.04^{cA}	0.39 ± 0.06^{cA}
	Combined	1.43 ± 0.09^{aA}	1.44 ± 0.12^{aA}	0.60 ± 0.21^{bA}	0.68 ± 0.06^{bA}	1.76 ± 0.22^{aA}	1.26 ± 0.12^{aA}	0.21 ± 0.03^{cA}	0.46 ± 0.04^{bA}
Respiration rate on day five, μg^{-1} C g^{-1} soil hr^{-1}	0 to 10	1.35 ± 0.04^{aA}	1.38 ± 0.13^{aA}	0.39 ± 0.02^{bA}	0.52 ± 0.03^{bA}	1.98 ± 0.33^{aA}	1.49 ± 0.09^{aA}	0.26 ± 0.01^{abA}	0.52 ± 0.05^{bA}
	10 to 20	1.41 ± 0.20^{aA}	1.35 ± 0.17^{aA}	0.37 ± 0.12^{bA}	0.68 ± 0.07^{bA}	1.46 ± 0.20^{abA}	1.18 ± 0.22^{aA}	0.20 ± 0.02^{bA}	0.39 ± 0.06^{bA}
	Combined	1.38 ± 0.09^{aA}	1.36 ± 0.10^{aA}	0.38 ± 0.06^{bA}	0.60 ± 0.05^{cA}	1.76 ± 0.22^{aA}	1.34 ± 0.12^{aA}	0.22 ± 0.02^{bA}	0.46 ± 0.04^{cA}
Mean respiration rate, μg^{-1} C g^{-1} soil hr^{-1}	0 to 10	1.42 ± 0.04^{aA}	1.44 ± 0.16^{abA}	0.61 ± 0.19^{bcA}	0.55 ± 0.03^{cA}	2.00 ± 0.32^{aA}	1.32 ± 0.08^{aA}	0.23 ± 0.03^{acA}	0.50 ± 0.07^{cA}
	10 to 20	1.39 ± 0.19^{aA}	1.36 ± 0.16^{aA}	0.37 ± 0.13^{bA}	0.73 ± 0.09^{bA}	1.61 ± 0.10^{abA}	0.94 ± 0.31^{aA}	0.32 ± 0.10^{bA}	0.36 ± 0.06^{bA}
	Combined	1.40 ± 0.09^{aA}	1.40 ± 0.11^{aA}	0.49 ± 0.12^{bA}	0.64 ± 0.06^{bA}	1.83 ± 0.19^{aA}	1.13 ± 0.16^{aA}	0.28 ± 0.06^{cA}	0.43 ± 0.05^{bA}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Upper case letters indicate contamination treatment means per amendment treatment are significantly different across that depth at $P < 0.05$

Table 2.9 Aerobic respiratory ratios measured in soil samples incubated without aeration

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Respiratory ratio on day two, moles CO_2/moles O_2	0 to 10	0.81 ± 0.19^{abA}	0.62 ± 0.13^{aA}	0.73 ± 0.21^{abA}	1.48 ± 0.31^{bA}	0.76 ± 0.11^{aA}	0.44 ± 0.04^{aA}	0.38 ± 0.16^{aA}	0.38 ± 0.09^{aB}
	10 to 20	0.70 ± 0.14^{aA}	0.64 ± 0.13^{aA}	0.54 ± 0.14^{aA}	0.99 ± 0.19^{aA}	0.58 ± 0.14^{abA}	0.62 ± 0.19^{aA}	0.15 ± 0.06^{bA}	0.31 ± 0.06^{abB}
	Combined	0.75 ± 0.11^{aA}	0.63 ± 0.08^{aA}	0.64 ± 0.12^{aA}	1.24 ± 0.19^{bA}	0.68 ± 0.08^{aA}	0.53 ± 0.10^{acA}	0.25 ± 0.08^{bB}	0.34 ± 0.05^{bcB}
Respiratory ratio on day five	0 to 10	0.68 ± 0.04^{aA}	0.67 ± 0.06^{aA}	0.38 ± 0.02^{bA}	0.54 ± 0.02^{cA}	0.71 ± 0.03^{aA}	0.63 ± 0.02^{aA}	0.26 ± 0.07^{aA}	0.54 ± 0.06^{aA}
	10 to 20	0.69 ± 0.02^{aA}	0.71 ± 0.03^{aA}	0.38 ± 0.06^{bA}	0.53 ± 0.02^{bA}	0.66 ± 0.04^{abA}	0.65 ± 0.05^{aA}	0.26 ± 0.04^{bA}	0.44 ± 0.06^{bA}
	Combined	0.68 ± 0.02^{aA}	0.69 ± 0.03^{aA}	0.38 ± 0.03^{bA}	0.54 ± 0.01^{cA}	0.69 ± 0.02^{aA}	0.64 ± 0.03^{aA}	0.26 ± 0.03^{bA}	0.49 ± 0.04^{cA}

Mean ± standard error

Amendment treatments: C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

Lower case letters indicate amendment treatment means are significantly different for that depth at $P < 0.05$

Upper case letters indicate contamination treatment means per amendment treatment are significantly different across that depth at $P < 0.05$

Table 2.10 Spearman correlation coefficients for correlations between microbiological and vegetation parameters

Parameter	Fungi	Diversity	PHC degraders		Microbial biomass C	Respiration rate	Respiratory ratio	Vegetation cover
			Aromatic	Aliphatic				
Fungi								
Diversity	ns							
Aromatic PHC degraders	ns	0.46						
Aliphatic PHC degraders	ns	0.50	0.64					
Microbial biomass carbon (C)	0.48	0.40	ns	0.43				
Respiration rate	0.39	ns	ns	0.33	0.64			
Respiratory ratio	0.34	0.42	0.35	0.40	0.68	0.78		
Vegetation cover	0.36	ns	ns	0.31	0.60	0.46	0.51	
Root biomass	ns	0.28	ns	0.30	0.63	0.60	0.65	0.78

ns = not significant at $P < 0.05$

Table 2.11 Spearman correlation coefficients for loss of PHCs from contaminated soils

Microbiological Parameter	Total extractable hydrocarbons	Fraction 2 PHCs	Fraction 3 PHCs	Fraction 4 PHCs
Aliphatic petroleum hydrocarbon (PHC) degrading microorganisms	0.42	0.51	0.42	ns
Microbial biomass carbon	0.57	0.62	0.58	0.49
Mean aerobic respiration rate	0.76	0.84	0.79	0.59
Respiratory ratio	0.74	0.80	0.77	0.61
Vegetation cover	0.75	0.76	0.77	0.73
Root biomass	0.76	0.72	0.79	0.74

Table 2.12 Pearson parametric correlation coefficients for correlations between vegetation parameters and remaining parameters

Correlating Parameter	Spearman test r_s	Pearson parametric test r	 r^2
Correlations with vegetation cover			
Fungi	0.36	0.27	0.07
Aliphatic petroleum hydrocarbon (PHC) degrading microorganisms	0.31	0.31	0.09
Microbial biomass carbon	0.60	0.41	0.17
Mean respiration rate	0.46	0.49	0.24
Day five respiratory ratio	0.51	0.51	0.26
Total extractable hydrocarbon (TEH) decrease	0.75	0.83	0.69
Fraction 2 ($>C_{10}$ to C_{16}) PHC decrease	0.76	0.83	0.69
Fraction 3 ($>C_{16}$ to C_{34}) PHC decrease	0.77	0.84	0.71
Fraction 4 ($>C_{34}$) PHC decrease	0.73	0.78	0.61
Root biomass	0.78	0.59	0.35
Correlations with root biomass			
Fungi	0.28	0.25	
Aliphatic PHC degrading microorganisms	0.30	0.24	
Microbial biomass carbon	0.63	0.37	0.14
Mean respiration rate	0.60	0.56	0.31
Day five respiratory ratio	0.65	0.54	0.29
Total TEH decrease	0.76	0.80	0.64
Fraction 2 PHC decrease	0.72	0.68	0.46
Fraction 3 PHC decrease	0.79	0.79	0.63
Fraction 4 PHC decrease	0.74	0.75	0.56

Underlined correlations are significant at $P < 0.05$

Table 2.13 Pearson parametric correlation coefficients for correlations between PHC decrease in contaminated soils and microbiological parameters

Correlating parameter	Spearman test r_s	Pearson parametric test r	 r^2
Correlations with decrease in total extractable hydrocarbons (TEH)			
Aliphatic PHC degrading microorganisms	0.42	0.36	0.13
Microbial biomass carbon	0.57	0.62	0.39
Mean respiration rate	0.76	0.75	0.56
Day five respiratory ratio	0.74	0.66	0.44

Table 2.13 continued

Correlating parameter	Spearman test	Pearson parametric test	
	r_s	r	r^2
Correlations with decrease in fraction 2 ($>C_{10}$ to C_{16}) petroleum hydrocarbon (PHCs)			
Aliphatic PHC degrading microorganisms	$\underline{0.51}$	$\underline{0.49}$	0.24
Microbial biomass carbon	$\underline{0.62}$	$\underline{0.72}$	0.52
Mean respiration rate	$\underline{0.84}$	$\underline{0.81}$	0.65
Day five respiratory ratio	$\underline{0.80}$	$\underline{0.73}$	0.54
Correlations with decrease in fraction 3 ($>C_{16}$ to C_{34}) PHCs			
Aliphatic PHC degrading microorganisms	$\underline{0.42}$	$\underline{0.38}$	0.14
Microbial biomass carbon	$\underline{0.58}$	$\underline{0.65}$	0.43
Mean respiration rate	$\underline{0.79}$	$\underline{0.80}$	0.63
Day five respiratory ratio	$\underline{0.77}$	$\underline{0.69}$	0.47
Correlations with decrease in fraction 4 ($>C_{34}$) PHCs			
Aliphatic PHC degrading microorganisms	0.33	0.34	
Microbial biomass carbon	$\underline{0.49}$	$\underline{0.53}$	0.28
Mean respiration rate	$\underline{0.59}$	$\underline{0.60}$	0.36
Day five respiratory ratio	$\underline{0.61}$	$\underline{0.53}$	0.28

Underlined correlations are significant at $P < 0.05$

Table 2.14 Pearson parametric correlation coefficients for combined contamination and amendment treatments

Correlating parameters	Sampling depth, cm	Spearman test	Pearson parametric test	
		r_s	r	r^2
Mean respiration rate vs day five respiratory ratio	Combined	$\underline{0.78}$	$\underline{0.74}$	0.55
Correlations with fungi				
Aerobic heterotrophic microorganisms for compost-only amendment treatment	0 to 10	$\underline{0.79}$	$\underline{0.783}$	0.61
	10 to 20	$\underline{0.81}$	$\underline{0.577}$	0.33
	Combined	$\underline{0.78}$	$\underline{0.670}$	0.45
Microbial biomass carbon	Combined	$\underline{0.48}$	$\underline{0.67}$	0.45
Mean respiration rate	Combined	$\underline{0.39}$	$\underline{0.47}$	0.22
Day five respiratory ratio	Combined	$\underline{0.34}$	$\underline{0.32}$	0.10
Correlations with aliphatic petroleum hydrocarbon (PHC) degraders				
Shannon-Weaver diversity index for colony morphology	0 to 10	0.13	0.15	
	10 to 20	$\underline{0.50}$	$\underline{0.37}$	0.14
	Combined	$\underline{0.35}$	$\underline{0.25}$	0.06

Table 2.14 continued

Correlating parameter	Sampling depth, cm	Spearman test	Pearson parametric test	
		r_s	r	r^2
Correlations with aliphatic PHC degraders				
Microbial biomass carbon	Combined	0.43	0.31	0.10
Mean respiration rate	Combined	0.33	0.26	0.07
Day five respiratory ratio	Combined	0.40	0.33	0.11
Correlations with aromatic PHC degrading microorganisms				
Aliphatic PHC degraders	Combined	0.64	0.23	
Microbial biomass carbon	Combined	0.27	0.12	
Day five respiratory ratio	Combined	0.35	0.25	
Colony morphological diversity index	0 to 10	0.19	0.21	
	10 to 20	0.46	0.43	0.18
	Combined	0.33	0.31	0.09
Correlations with colony morphological diversity index				
Colony morphological richness per plate at 10^{-5} soil dilution	0 to 10	0.69	0.76	0.58
	10 to 20	0.81	0.83	0.68
	Combined	0.76	0.79	0.63
Mean respiration rate	0 to 10	0.18	0.17	
	10 to 20	0.33	0.35	
	Combined	0.31	0.28	0.08
Day five respiratory ratio	0 to 10	0.01	0.22	
	10 to 20	0.42	0.46	0.21
	Combined	0.24	0.36	0.13
Correlations with microbial biomass carbon				
Colony morphological diversity index	0 to 10	-0.09	0.00	
	10 to 20	0.40	0.30	
	Combined	0.16	0.18	
Mean respiration rate	Combined	0.64	0.52	0.27
Day five respiratory ratio	Combined	0.68	0.60	0.36

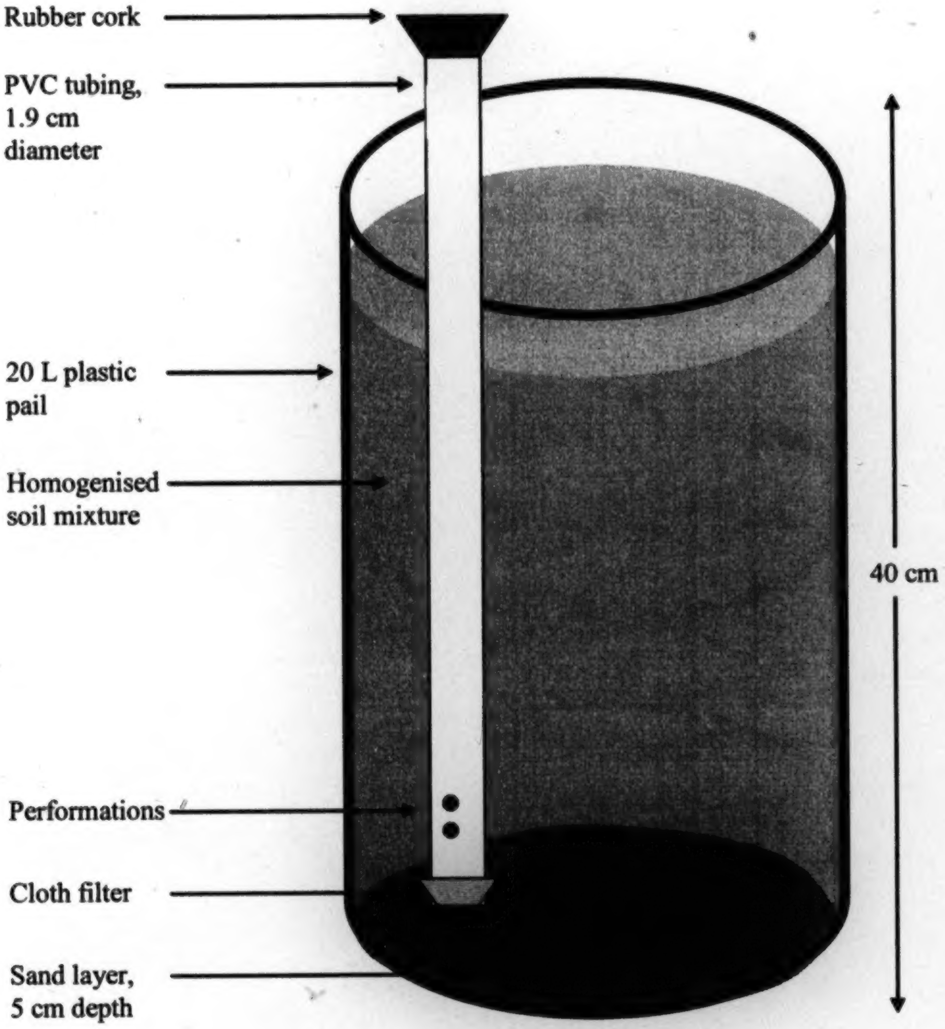


Figure 2.1 Pails for simulated in situ bioremediation experiment.

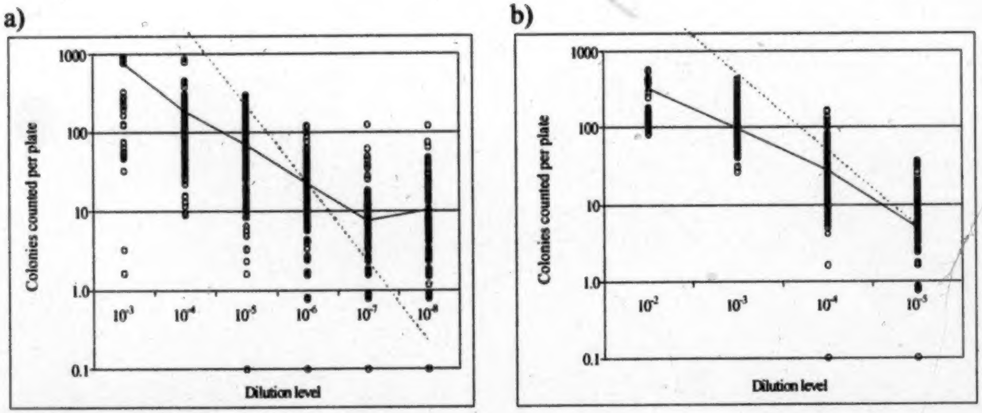


Figure 2.2 Soil dilution effect on combined a) plate count agar counts and b) Martin's media (fungi) counts

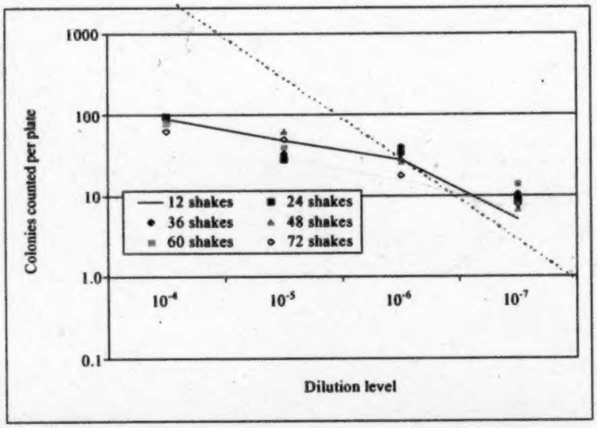


Figure 2.3 Effects of shaking on soil dilution effect for plate count agar counts of aerobic heterotrophic microorganisms
Dashed line is theoretical slope, solid line is calculated slope

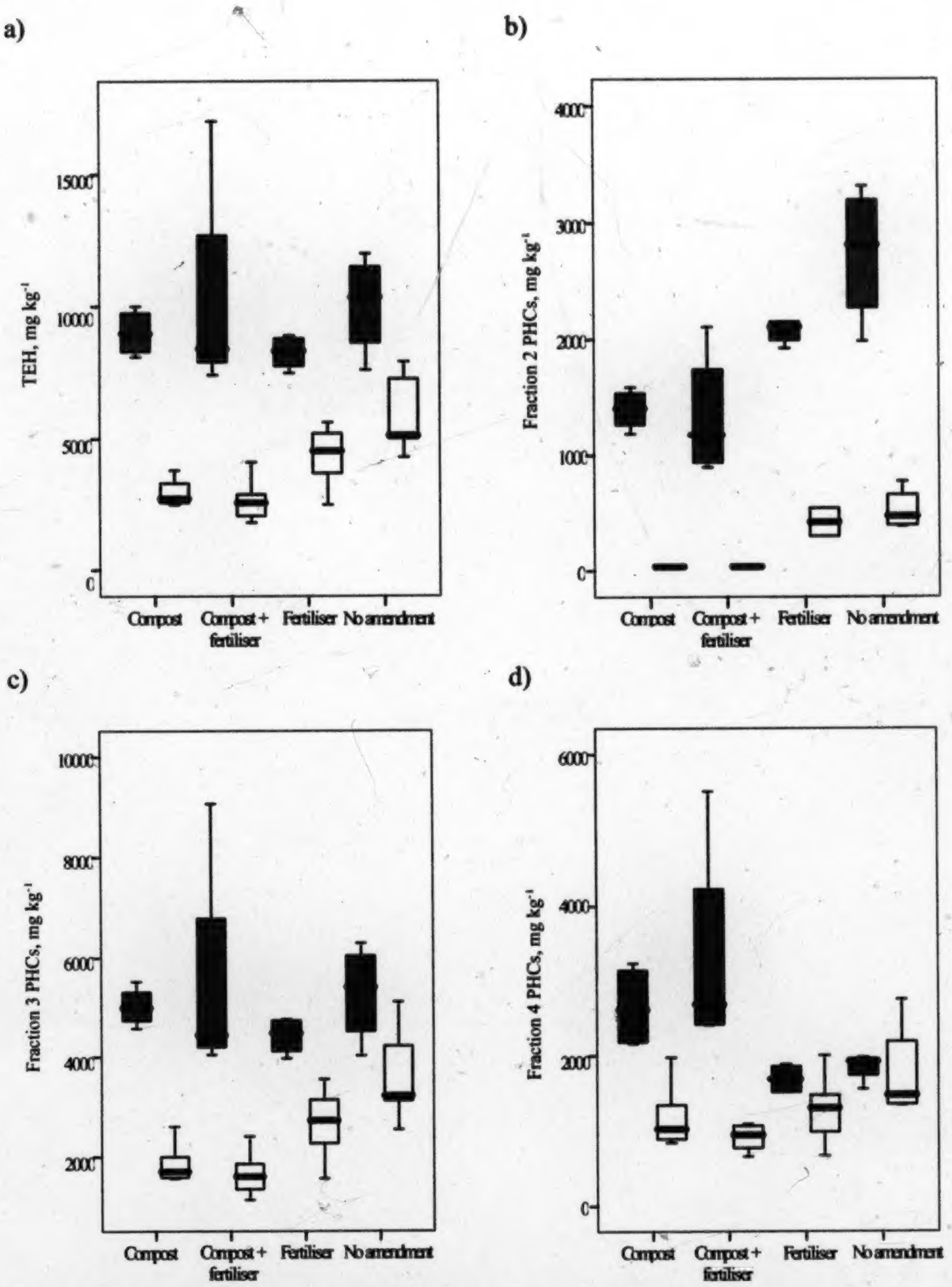


Figure 2.4 Boxplots of a) total extractable hydrocarbon content and b) fraction 2, c) fraction 3 and d) fraction 4 petroleum hydrocarbon (PHC) concentrations in contaminated soils of combined sampling depths
Grey = results from start of field trial in June 2003; white = results from end of field trial in August 2004

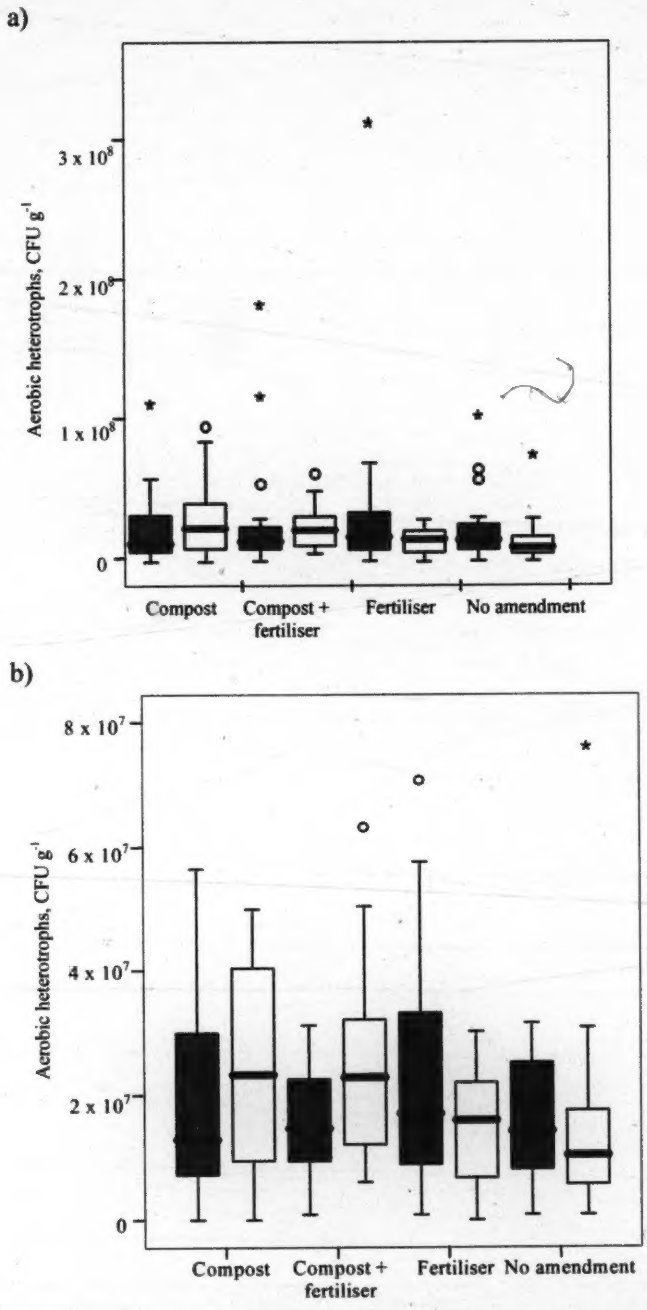
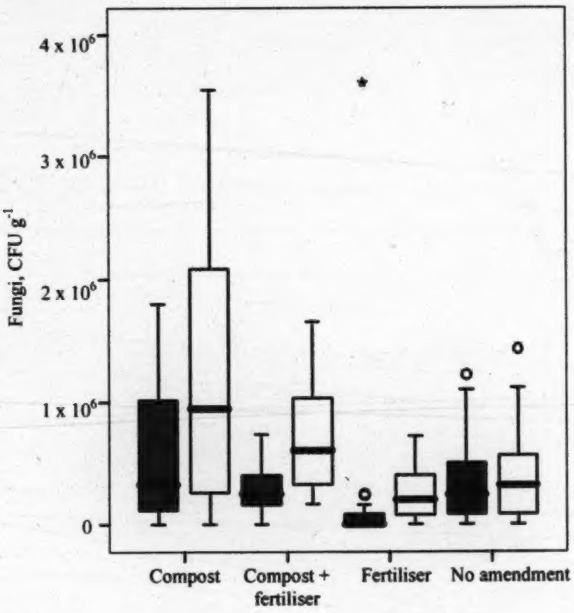


Figure 2.5 Plate count data for aerobic heterotrophic microorganisms at combined sampling depths a) for all data and b) with extreme cases (stars) and outliers (circles) removed
Grey = crude oil treatment, white = no contamination treatment

a)



b)

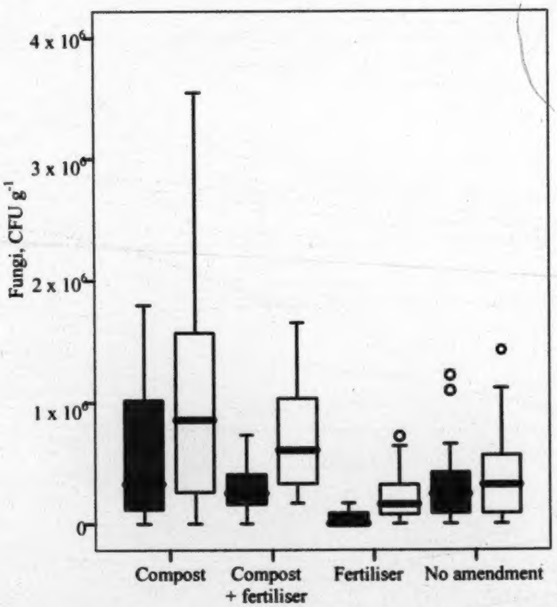
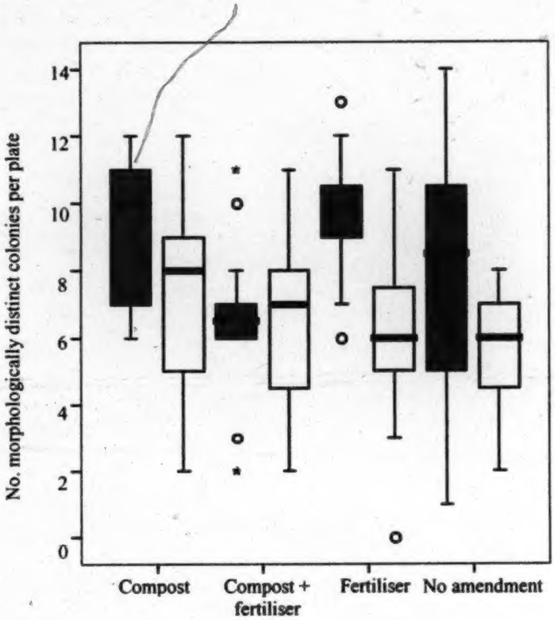


Figure 2.6 Plate count data for viable fungi at combined sampling depths a) for all data and b) with extreme cases (stars) and outliers (circles) removed
Grey = crude oil treatment, white = no contamination treatment

a)



b)

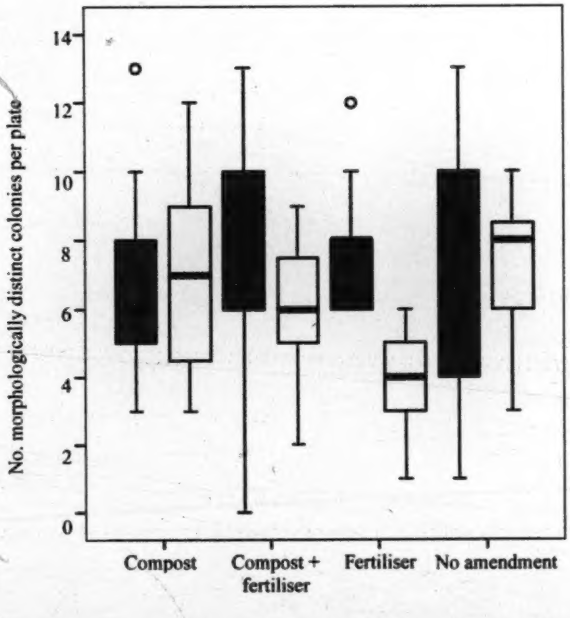
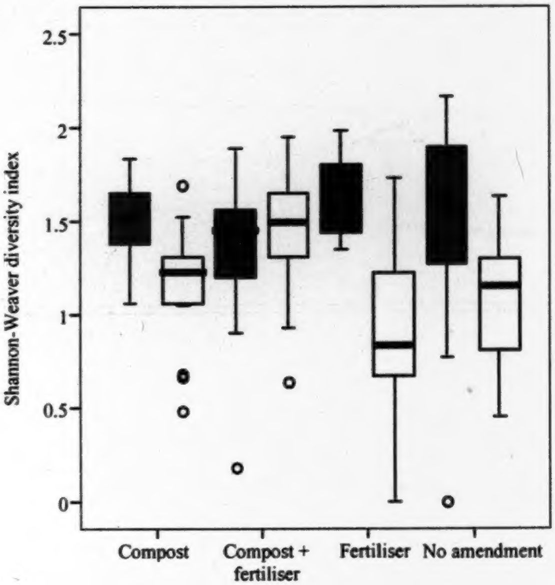


Figure 2.7 Colony morphological richness on plate count agar at 10^5 soil dilution, measured at a) 0 to 10 cm sampling depth and b) 10 to 20 cm sampling depth
Grey = crude oil treatment, white = no contamination treatment
Stars = extreme cases, circles = outliers

a)



b)

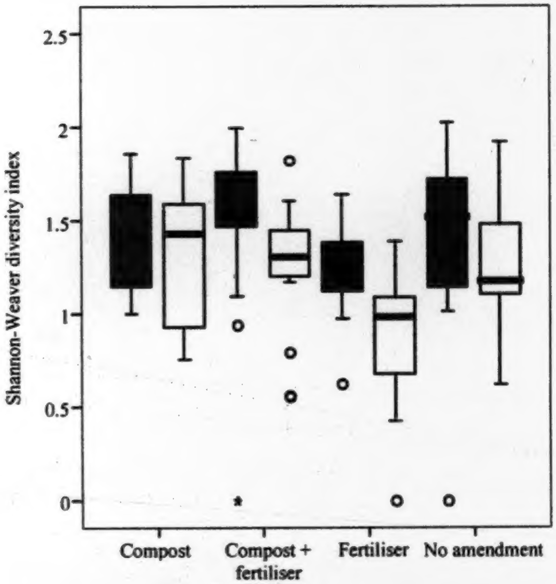


Figure 2.8 Colony morphological diversity index on plate count agar at 10^5 soil dilution, measured at a) 0 to 10 cm sampling depth and b) 10 to 20 sampling depth
Grey = crude oil treatment, white = no contamination treatment
Stars = extreme cases, circles = outliers

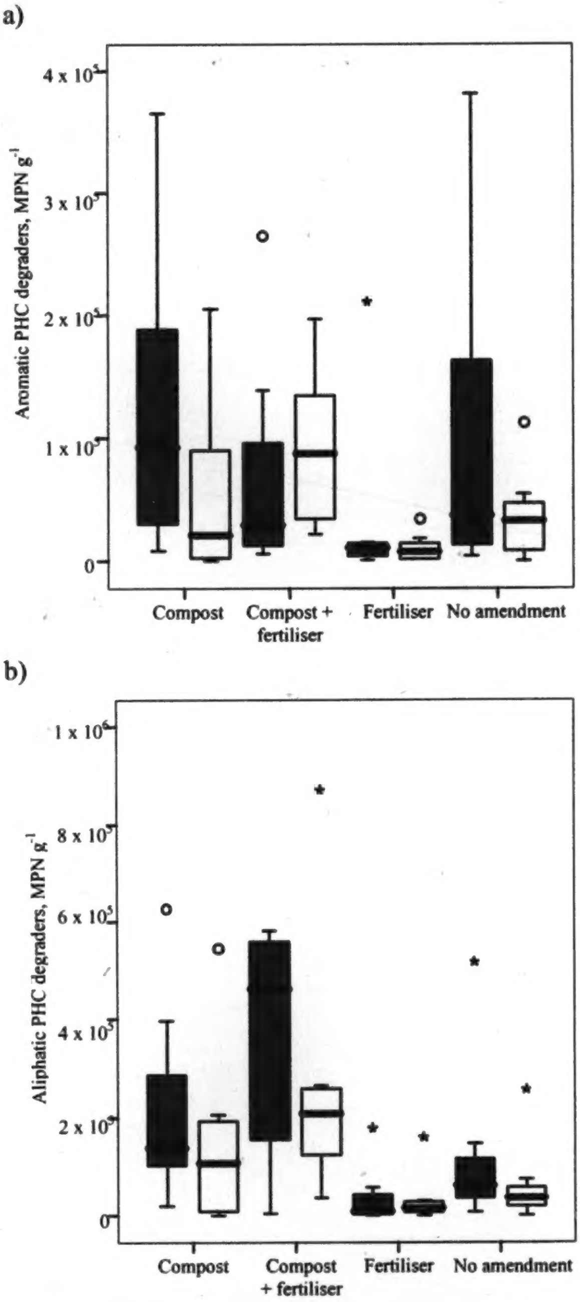


Figure 2.9 96-well plate results for a) aromatic and b) aliphatic petroleum hydrocarbon (PHC) degrading microorganisms at combined sampling depths
Grey = crude oil treatment, white = no contamination treatment
Stars = extreme cases, circles = outliers

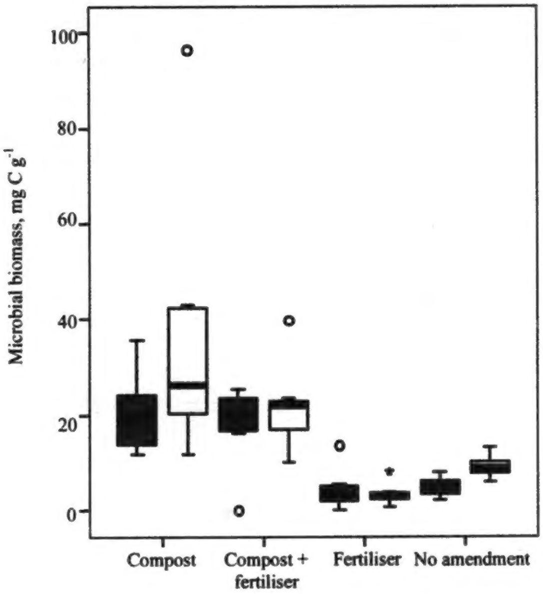


Figure 2.10 Microbial biomass carbon at combined sampling depths

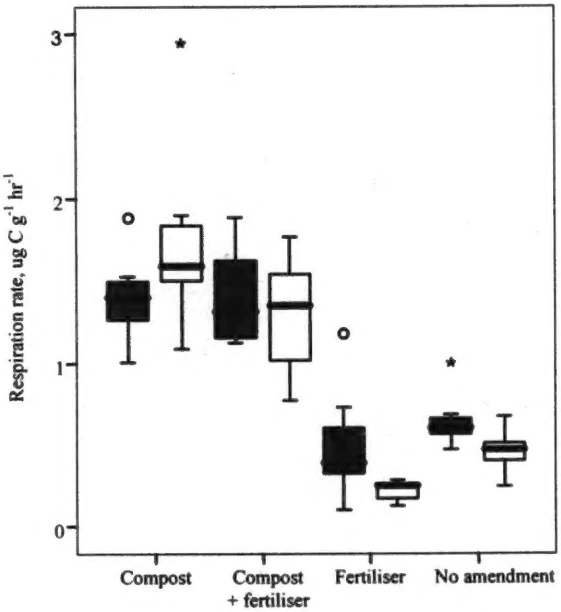
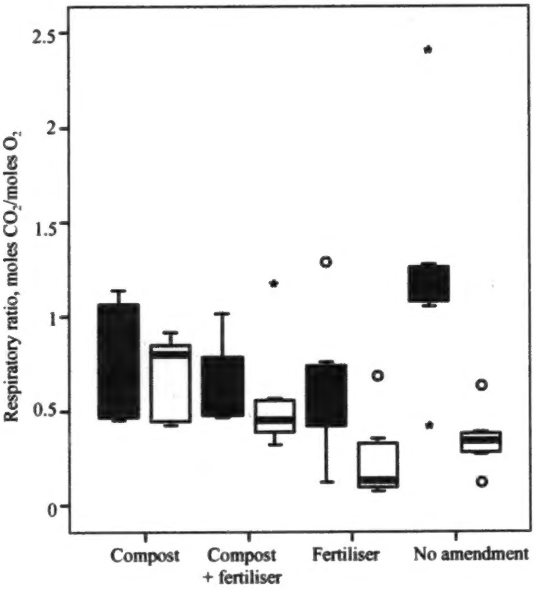


Figure 2.11 Mean aerobic respiration rate for combined sampling depths
Grey = crude oil treatment, white = no contamination treatment
Stars = extreme cases, circles = outliers

a)



b)

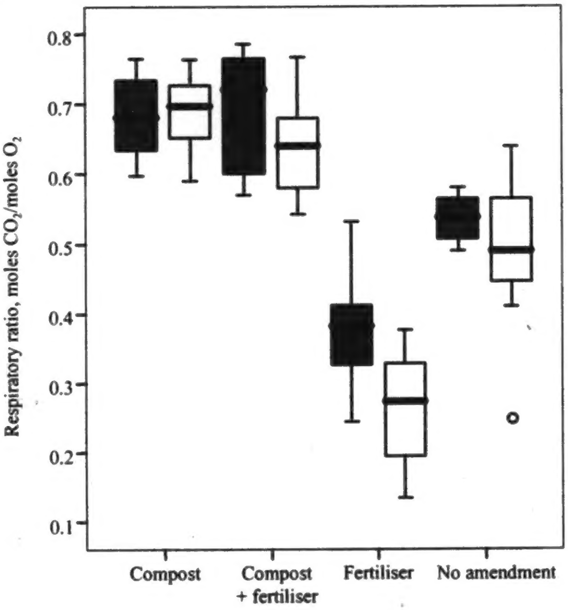


Figure 2.12 Respiratory ratios on a) day two and b) day five of incubation for combined sampling depths
Grey = crude oil treatment, white = no contamination treatment
Stars = extreme cases, circles = outliers

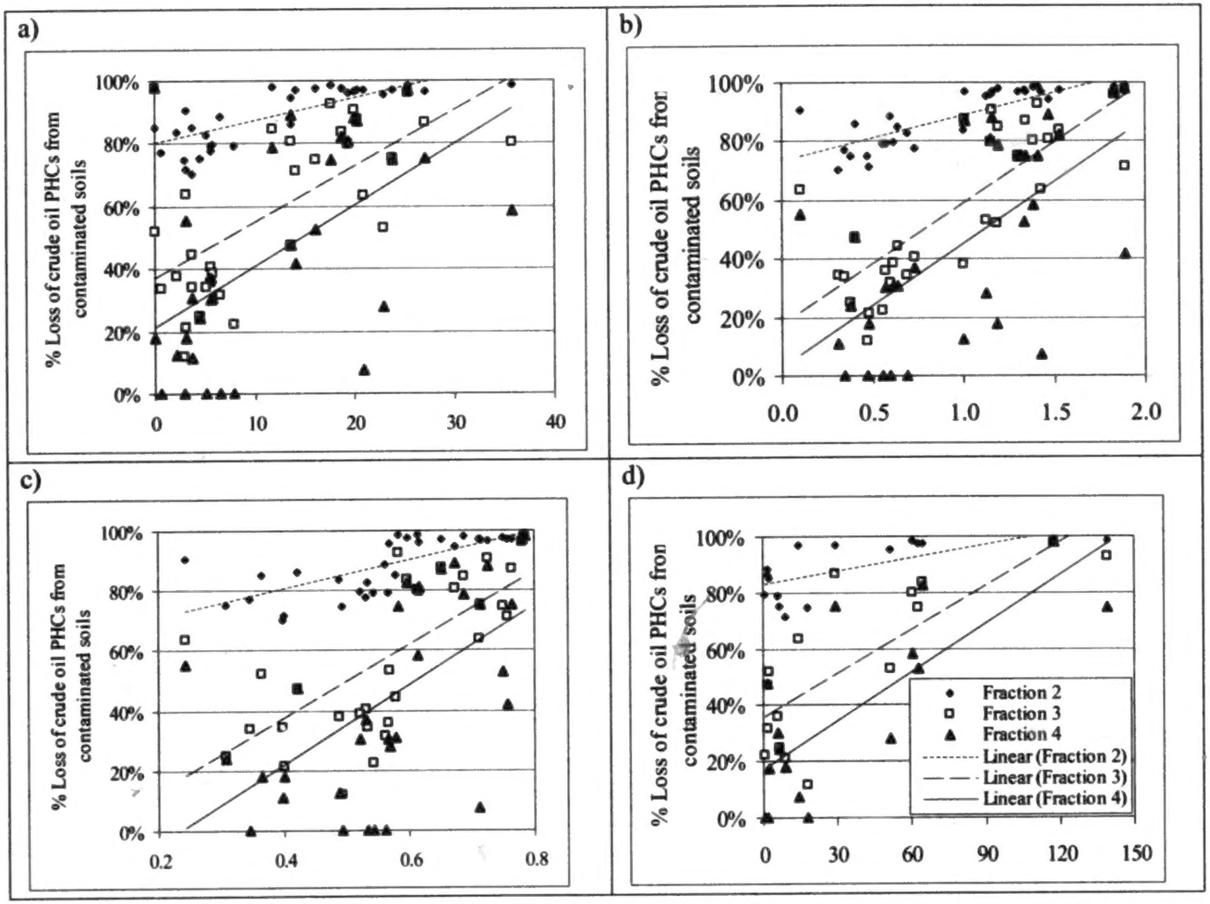


Figure 2.13 Correlations between petroleum hydrocarbon (PHC) loss per fraction and a) microbial biomass carbon (mg C g^{-1}), b) mean aerobic respiration rate (μg C g^{-1} hr^{-1}), c) respiratory ratio measured on day five of incubation, and d) root biomass (Mg m^{-3})

3. BIOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOILS: A SYNTHESIS

3.1 RESEARCH FINDINGS

Studies of petroleum hydrocarbon (PHC) contamination in soils have focused upon use of inorganic fertilisers, oxygenation and surfactants for bioremediation. Organic amendments such as compost are receiving more attention. Few experiments using compost in PHC bioremediation have separated degradation of total extractable hydrocarbons (TEHs) and the larger, recalcitrant PHCs classified as fraction 3 and 4 by the Canadian Council of Ministers of the Environment (CCME). In particular, the impact of compost and fertilisers on microbial population size and activity in remediated soils has been neglected. Most of the research has been conducted in the laboratory and not in the field, particularly in the cool climate of western Canada.

A study was done to determine effects of municipal solid waste-biosolids co-compost and inorganic fertiliser on bacterial and fungal population size, respiration and biomass in soil contaminated with crude oil under in situ conditions. Hydrocarbon-degrading microorganisms were enumerated and bacterial richness and diversity estimated to determine if crude oil contamination and amendments affect soil microbial communities. These microbiological parameters were correlated with disappearance of PHCs from contaminated soil to measure the impact of microbiological properties on soil bioremediation.

This study found that crude oil contamination decreased microbial biomass and fungal populations, while increasing numbers of PHC-degrading microorganisms, community richness and diversity, and aerobic respiration. Application of ammonium sulphate ($(NH_4)_2SO_4$) fertiliser decreased soil carbon dioxide (CO_2) respiration and fungi, and had little effect upon remaining soil microbiological properties. Compost amendment, with and without $(NH_4)_2SO_4$ amendment, greatly increased microbial biomass, populations of fungi and PHC-degrading microorganisms and soil respiration. Graham (2005) reported that compost improved bulk density and total organic carbon (TOC) content and added macro and micronutrients to affected soils in this experiment. Increases in soil microbial populations and activity in this study are attributed to these changes in soil physical and chemical properties. PHC decreases in all four CCME fractions were correlated with aliphatic PHC degrading population size, microbial biomass carbon, CO_2 respiration rate, respiratory ratio, vegetation cover and root biomass. These correlations were stronger for fraction 2 ($>C_{10}$ to C_{16}) than for fraction 3 ($>C_{16}$ to C_{34}) or fraction 4 ($>C_{34}$).

Compost amendment proved to be an effective amendment for crude oil bioremediation in the field. Application of the results of this experiment must also be tempered with the knowledge that maximum PHC degradation in the field occurs at the shallow depths sampled (Van Hamme et al. 2003), due to increased oxygen (O_2) availability and higher volatilisation rates (Song and Bartha 1990; Van Hamme et al. 2003), and bioremediation rates would thus be lower for deeper soils.

3.2 FUTURE RESEARCH DIRECTIONS

Because PHC degradation follows first-order kinetics, most of the degradation of PHCs measured in this experiment would have occurred in the first few weeks or months of the first field season (Salanitro 2001; Van Hamme et al. 2003). Microbiological sampling was conducted 14 months after the start of the experiment, well after most of the microbial degradation of crude oil would have occurred; microbial populations at this point were likely moving towards equilibrium, as were corresponding soil properties such as nutrient concentrations. Initial characterisation and monitoring of the soil microbial community is recommended for future bioremediation trials to evaluate changes in microbial populations over time.

The effect of weed invasion cannot be calculated due to the lack of non-vegetated controls. Roots in composted-amended soils extended to the bottom 20 cm sampled and lower (Graham 2005), so the rhizosphere effect may have been responsible for increasing microbial metabolism of PHCs. Almost no vegetation grew in contaminated soils without compost amendment, however, indicating that ultimately compost amendment was responsible for ameliorating PHC contamination; compost was required to reduce soil phytotoxicity for weeds to establish, so any effect of vegetation is a direct result of the effect of compost. Trials comparing compost-amended contaminated soils with and without vegetation are recommended to distinguish between the effects of compost and the effects of vegetation on PHC biodegradation.

The significantly higher leachate production from contaminated soils indicates crude oil increased soil hydrophobicity, reducing moisture retention. Water uptake by vegetation would also reduce leaching. Crude oil could be smelled in leachate from pails not amended with compost at the end of the field trial, suggesting that, although hydrophobic, PHCs may have been removed from both contaminated and non-contaminated soils by mass movement with water. Leachate production did not differ significantly between amendment treatments, and values appeared lower for compost-amended soils than for fertiliser only and control treatments; the great decreases in PHC concentrations observed in compost treatments compared to control treatments were therefore not caused by leaching. Chaîneau et al. (2003) reported similar results from a windrow experiment in which leachate was monitored for 480 days, over which time approximately 700 mm of precipitation fell. Hydrocarbons leached from clay soil of 1.8% crude oil contamination represented 0.02% of the initial hydrocarbon load, with most of these hydrocarbons leached during the first 90 days of the experiment. Soil hydrophobicity analysis and leachate monitoring for PHC in future field trials is recommended to determine if compost application ameliorates oil-induced hydrophobicity.

Some of the drop in PHC concentrations in compost-amended, contaminated soil may be due to sorption to compost humic material and to leaching, but the corresponding increases in CO_2 production, microbial biomass, and numbers of aromatic and aliphatic PHC degrading microorganisms indicate that PHCs were indeed being metabolised. CCME fraction 4 PHCs present in compost at the start of the experiment were lost over the field trial, a result that cannot be attributed to sorption. Partial breakdown of fraction 4 to fraction 3 size may be responsible for the small increases in fraction 3 concentrations, accompanied by drops in fraction 4 content, noted in some non-contaminated treatments amended with compost. In this study PHC content was measured by TEH content; differing analytical methods may be applied to separate total and available PHC concentrations (Kästner et al. 1995; Kästner and Mahro 1996).

Oxygen concentrations measured in situ on the day of sampling were equal to those in the atmosphere, indicating that anaerobic conditions did not exist at the end of the field experiment. Without continuous monitoring of concentrations from the beginning of the trial, however, the role of anaerobic respiration in PHC biodegradation cannot be estimated. Concentrations of the alternative electron acceptors manganese (Mn^{+2}) and sulphate (SO_4^{-2}) did not significantly differ between contamination treatments, and no methane (CH_4) production was observed during aerobic incubation. Concentrations of nitrate (NO_3^-), which is thermodynamically preferred to SO_4^{-2} as an alternate electron acceptor (Fathepure and Tiedje 1998), were significantly lowered in contaminated treatments, but this was attributed to inhibition of nitrification by crude oil (Hyman et al. 1988; Chang et al. 2002). Although variation in these parameters was high due to differing amendment treatments, vegetation growth and leachate production, these results suggest no significant anaerobic degradation of PHCs occurred. Anaerobic conditions are unlikely to develop or persist at the shallow depths studied. Since leachate and standing water was pumped out of the pails immediately following precipitation events, soils were not saturated for significant periods of time and air diffusion therefore not limited; these conditions may differ at contaminated field sites, however.

Relative increases in aromatic and aliphatic PHC degrading microorganisms resulting from crude oil contamination were greater than those in aerobic heterotrophic bacteria, fungi, or microbial biomass carbon, indicating that microorganisms capable of metabolising PHCs became more dominant. Increases in colony morphological richness and diversity also indicate a change in the composition of the microbial community. Compost amendment of contaminated soils supported increases in PHC degrading microorganisms, whereas fertiliser only amendment did not. The greater increases, and higher statistical significance, of aliphatic PHC degrading microbial populations compared to aromatic PHC degrading microorganisms may be attributed in part to the composition and biodegradability of crude oil, which typically contains more aliphatic than aromatic PHCs (Bailey et al. 1973; Westlake et al. 1974; Salanitro 2001). The saturate fraction of crude oil is the most amenable to microbial attack, causing a relatively greater response of aliphatic PHC degrading microorganisms. Analysis of six crude oils from wells in Saskatchewan yielded saturate fractions of 19.1 to 47.1% and aromatic fractions of 37.5 to 43.4%, with saturate:aromatic ratios of 0.44, 0.77, 0.88, 0.91, 1.03 and 1.26 dropping with proximity to freshwater and thus exposure to microbial attack (Bailey et al. 1973). Analysis of crude oil composition is recommended for future research to separate degradation of aromatic and non-aromatic PHCs.

Ammonium sulphate fertiliser acidified soil greatly, a result observed in other studies using ammonium (NH_4^+) fertilisers. Ammonium sulphate was chosen based upon the work of Brook et al. (2001), in which $(NH_4)_2SO_4$ yielded higher hydrocarbon degradation rates than ammonium nitrate (NH_4NO_3), potassium nitrate (KNO_3), and urea fertilisers. The carbon to nitrogen (C:N) ratios tested were 40:1 and 20:1, a great deal higher than the 10:1 ratio used in this study, and the test soil more alkaline at pH 7.9. Phosphorus was also added to adjust the soil carbon to phosphorus (C:P) ratio to 200:1 (Brook et al. 2001), whereas phosphorus (P) was not added in this study and concentrations were not measured in contaminated soils.

PHC-degrading microorganisms prefer neutral to slightly alkaline pH, and the lowered bioremediation rates observed in fertiliser amended soils were therefore attributed to acidification. Lewis et al. (1984) reported sharp drops in *Flavobacterium* transformation of insecticide and herbicide as culture media pH dropped from 7.2 to 3.8. Fungi are not as vulnerable to acidification, however (Trevors 1998), and often acidify their environments by producing organic acids (Lewis et al. 1984). Compost amendment buffered pH changes in compost + fertiliser treatments, ameliorating this effect. Differences between compost and inorganic fertiliser should therefore be tested with different inorganic fertilisers, in which soil acidification would play little or no effect. Analysis of soil P content is also recommended.

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APPENDIX

Table A1 Summary of CCME Canada-Wide Standards Tier 1 levels for petroleum hydrocarbons in surface soil

Land use	Soil texture	Fraction 1 C_6 to C_{10}	Fraction 2 $>C_{10}$ to C_{16}	Fraction 3 $>C_{16}$ to C_{34}	Fraction 4 $>C_{34}$
Agricultural	Coarse-grained	130	450 (150[a])	400	2800
	Fine-grained	260 (180[b])	900 (250[b])	800	5600
Residential / parkland	Coarse-grained	30	150	400	2800
	Fine-grained	260 (180[b])	900 (250[b])	800	5600
Commercial	Coarse-grained	310 (230[a])	760 (150[a])	1700	3300
	Fine-grained	660 (180[b])	1500 (250[b])	2500	6600
Industrial	Coarse-grained	310 (230[a])	760 (150[a])	1700	3300
	Fine-grained	660 (180[b])	1500 (250[b])	2500	6600

[a] Where applicable, for protection against contaminated groundwater discharge to an adjacent surface water body

[b] Where applicable, for protection of potable groundwater

Values in mg kg^{-1}

Taken directly from Canadian Council for Ministers of the Environment (CCME) 2001

Petroleum hydrocarbon (PHC) concentrations in mg kg^{-1}

Table A2 Gibbs free energies of toluene and naphthalene metabolism

Reaction	$\Delta G^{0\prime}$ kcal $mole^{-1}$
Toluene metabolism at 1 M, 25 °C and pH 7.0	
$C_7H_8 + 9\ O_2 + 3\ H_2O \rightarrow 7\ HCO_3^- + 7\ H^+$	-910
$C_7H_8 + 7.2\ NO_3^- + 0.5\ H^+ \rightarrow 7\ HCO_3^- + 3.6\ N_2 + 0.6\ H_2O$	-847
$C_7H_8 + 36\ Fe^{+3} + 21\ H_2O \rightarrow 7\ HCO_3^- + 36\ Fe^{+2} + 43\ H^+$	-878
$C_7H_8 + 4.5\ SO_4^{-2} + 3\ H_2O \rightarrow 7\ HCO_3^- + 2.25\ HS^- + 2.25\ H_2S + 0.25\ H^+$	-54.7
$C_7H_8 + 5\ H_2O \rightarrow 4.5\ CH_4 + 2.5\ HCO_3^- + 2.5\ H^+$	-31.2

Table A2 continued

Reaction	$\Delta G^{0'}$ kcal $mole^{-1}$
Naphthalene metabolism at 25 °C and pH 7	
$C_{10}H_8 + 12\ O_2 \rightarrow 10\ CO_2 + 4\ H_2O$	-1221
$C_{10}H_8 + 24\ Mn^{+4} + 20\ H_2O \rightarrow 10\ CO_2 + 24\ Mn^{+2} + 48\ H^+$	-1683
$C_{10}H_8 + 48\ Fe^{+3} + 20\ H_2O \rightarrow 10\ CO_2 + 48\ Fe^{+2} + 48\ H^+$	-1177
$C_{10}H_8 + 9.6\ NO_3^- + 9.6\ H^+ \rightarrow 10\ CO_2 + 4.8\ N_2 + 8.8\ H_2O$	-1146
$C_{10}H_8 + 6\ SO_4^{-2} + 9\ H^+ \rightarrow 10\ CO_2 + 3\ HS^- + 3\ H_2S + 4\ H_2O$	-80
$C_{10}H_8 + 8\ H_2O \rightarrow 6\ CH_4 + 4\ CO_2$	-47.5

Source: Fathepure and Tiedje 1998

Table A3 Climate conditions at the research site relative to climate normals for the area

	Temperature (°C)		Rainfall (mm)		Snowfall (cm)
	Research site	Climate normals	Research site	Climate normals	Climate normals
2003					
June	14.8	15.5	55.9	87.1	0
July	18.2	17.5	91.7	91.7	0
August	17.5	16.6	116.6	68.9	0
September	11.0	11.3	9.1	42.3	1.5
October	6.5	5.6	10.7	10.5	7.8
November	-7.0	-4.1	1.3	1.9	17.9
December	-7.8	-9.6	0.0	0.8	22.3
2004					
January	-14.6	-11.7	0.0	1.3	24.5
February	*	-8.4	*	0.9	15.8
March	*	-2.6	*	2.1	16.8
April	*	5.5	*	13.1	13.4
May	9.5	11.7	20.3	45.1	3.5
June	15.2	15.5	19.3	87.1	0
July	17.4	17.5	129.5	91.7	0
August	15.1	16.6	35.3	68.9	0

Source: Environment Canada 2004

* Data not available

Taken directly from Graham 2005

Table A4 Weedy plant species invading in bioremediation experiment as of August 2004

Scientific name	Common name
Amaranthus retroflexus L.	Redroot pigweed
Artemisia absinthium L.	Absinth
Capsella bursa-pastoris (L.) Medik.	Shepherd's-purse
Chenopodium album L.	Lamb's-quarters
Crepis tectorum L.	Narrow-leaf hawk's beard
Echinochloa crus-galli (L.) Beauv.	Barnyard grass
Erodium cicutarium (L.) L'Hér. ex Ait.	Redstem stork's bill
Hordeum jubatum L.	Foxtail barley
Kochia scoparia (L.) Schrad.	Kochia or burning bush or Mexican fireweed
Matricaria discoidea DC. or *Matricaria matricarioides* auct. non (Less.) Porter	Disc mayweed or pineapple weed
Polygonum aviculare L.	Prostate knotweed
Polygonum convolvulus L.	Wild buckwheat or black bindweed
Portulaca oleracea L.	Purslane or little hogweed
Potentilla norvegica L.	Rough cinquefoil or Norwegian cinquefoil
Setaria viridis (L.) Beauv.	Green foxtail or green bristlegrass
Sonchus arvensis L.	Perennial sow-thistle or field sowthistle
Taraxacum officinale G.H. Weber ex Wiggers	Common dandelion

Compiled from Moss et al. 1983, White et al. 1993, Dunn and Blackshaw 1999, Godwin and Thorpe 2000, Bergstrom 2004, USDA NRCS 2004

Table A5 Characterisation of City of Edmonton municipal solid waste (MSW) and sewage biosolids compost relative to other MSW compost stocks and CCME guidelines, from Graham (2005)

	City of Edmonton MSW-biosolid compost	MSW compost literature values[1]	CCME limits	
	Mean[1] (range)	(range)	Class A	Class B
Total organic carbon (%)	19.9 (19.2 - 21.2)	(12.4 – 43.2)		
Total Kjeldahl nitrogen (%)	1.85 (1.84 - 1.86)	(0.45 – 3.1)		
Carbon to nitrogen ratio	11.2 (10.8 - 11.9)	(9.9 – 22)	< 25	
Moisture (%)	36.9 (35.4 – 37.8)	(25- 47.3)		
Nitrate (mg kg^{-1})	1866 (1730 - 1990)	16.5		
Ammonium (mg kg^{-1})	7.5 (7.1 - 7.7)	865		
pH	7.7 (7.6 - 7.7)	(6.3 – 8.9)		
Electrical conductivity (dS m^{-1})	16.7 (15.8 - 17.3)	(2.4 – 16)		
Sodium adsorption ratio	15.9 (15.1 - 16.3)			
Sodium (mg L^{-1})	2387 (2210 - 2490)			
Fecal coliforms (MPN g^{-1})	<3		<1000	
Salmonella (MPN $4g^{-1}$)	not detected		not detected	
Total extractable hydrocarbons (mg kg^{-1})	9033 (8400 – 10000)			
Arsenic (mg kg^{-1})	5 (4.6 - 5.4)	(0.9 – 52)	13	75
Cadmium (mg kg^{-1})	3 (2.9 - 3.1)*	(0.04 – 100)	3	20
Chromium (mg kg^{-1})	81.7 (77.2 - 85.3)	(2 – 366)	210	1060
Cobalt (mg kg^{-1})	6.3 (6 - 7)	(15-48)	34	150
Copper (mg kg^{-1})	200 (194 - 203)*	(28 – 630)	100	757
Lead (mg kg^{-1})	152 (147 - 157)*	(9 – 900)	150	500
Mercury (mg kg^{-1})	0.65 (0.59 - 0.69)	(0.5 – 21)	1	5
Molybdenum (mg kg^{-1})	3.7 (3 - 4)	(1.0 – 25)	5	20
Nickel (mg kg^{-1})	53 (49 - 56)	(0.76 – 200)	65	180
Selenium (mg kg^{-1})	0.5 (0.4 - 0.6)	(0.5 – 3.6)	2	14
Zinc (mg kg^{-1})	646 (632 - 665)*	(37.6 – 1650)	500	1850

[1] Sources: Gallardo-Lara and Nogales 1987, He et al. 1992, Iglesias-Jimenez and Alvarez 1993, Villar et al. 1993, He et al. 1995, Sikora and Yakovchenko 1996, Cuevas et al. 2000, Zhang et al. 2000, Marcote et al. 2002, Zaccheo et al. 2002

* exceeds Canadian Council of Ministers of the Environment (CCME) class A guidelines

Taken directly from Graham 2005

Table A6 Probability results of non-parametric t-test to detect differences between aerobic respiration parameters

	Sampling depth, cm	Crude oil contamination				No contamination			
		C	CF	F	NA	C	CF	F	NA
Difference between aerobic respiration rates on day two and day five	0 to 10	0.14	0.14	0.07	0.07	0.72	0.27	0.11	0.27
	10 to 20	0.07	0.46	0.72	0.07	0.11	0.07	0.14	0.72
	Combined	0.67	0.09	0.21	0.01	0.24	0.09	0.50	0.40
Difference between respiratory ratios on day two and day five	0 to 10	0.46	0.46	0.14	0.07	0.46	0.07	0.28	0.11
	10 to 20	1.00	0.46	0.46	0.14	0.41	0.72	0.20	0.07
	Combined	0.48	0.40	0.12	0.02	0.80	0.16	0.80	0.02

C = compost only, CF = compost + fertiliser, F = fertiliser only, NA = no amendment

Table A7 Probability results of non-parametric ANOVAs on effects of crude oil contamination and amendment

		Scheirer-Ray-Hare test			Kruskal Wallis test	
	Sampling depths (cm)	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Aerobic heterotrophs	0 to 10	0.80	0.30	0.57	0.80	0.01
	10 to 20	0.81	0.21	0.70	0.81	0.00
	Combined	0.74	0.52	0.41	0.74	0.08
Aerobic heterotrophs without extreme cases and outliers	0 to 10	1.00	0.23	0.63	0.92	0.00
	10 to 20	0.50	0.21	0.70	0.47	0.00
	Combined	0.63	0.54	0.42	0.66	0.07
Fungi	0 to 10	0.01	0.00	0.50	0.01	0.00
	10 to 20	0.00	0.07	0.98	0.00	0.00
	Combined	0.00	0.00	0.67	0.00	0.00

Table A7 continued

		Scheirer-Ray-Hare test			Kruskal Wallis test	
	Sampling depths (cm)	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Fungi without extreme cases and outliers	0 to 10	0.01	0.00	0.58	0.01	0.00
	10 to 20	0.00	0.04	0.86	0.00	0.00
	Combined	0.00	0.00	0.72	0.00	0.00
Aromatic PHC degraders	0 to 10	0.76	0.76	0.71	0.76	0.31
	10 to 20	0.39	0.39	0.93	0.39	0.03
	Combined	0.29	0.28	0.71	0.29	0.01
Aliphatic PHC degraders	0 to 10	0.47	0.53	0.97	0.47	0.09
	10 to 20	0.62	0.15	0.97	0.62	0.00
	Combined	0.27	0.06	0.97	0.26	0.00
Colony morphological richness	0 to 10	0.00	0.35	0.50	0.00	0.02
	10 to 20	0.00	0.62	0.24	0.00	0.15
	Combined	0.00	0.82	0.28	0.00	0.42
Colony morphological richness without extreme cases and outliers	0 to 10	0.00	0.25	0.44	0.00	0.01
	10 to 20	0.01	0.56	0.24	0.00	0.08
	Combined	0.00	0.84	0.32	0.00	0.42
Colony morphological diversity	0 to 10	0.00	0.85	0.18	0.00	0.50
	10 to 20	0.00	0.16	0.87	0.00	0.00
	Combined	0.00	0.26	0.40	0.00	0.01

Table A7 continued

		Scheirer-Ray-Hare test			Kruskal Wallis test	
	Sampling depths (cm)	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Colony morphological diversity without extreme cases or outliers	0 to 10	0.00	0.17	0.33	0.00	0.28
	10 to 20	0.00	0.73	0.16	0.00	0.00
	Combined	0.00	0.15	0.83	0.00	0.00
Microbial biomass carbon	0 to 10	0.17	0.08	0.94	0.14	0.00
	10 to 20	0.71	0.04	0.91	0.71	0.00
	Combined	0.21	0.00	0.94	0.21	0.00
Aerobic respiration rate measured on day two of incubation	0 to 10	0.55	0.08	0.82	0.74	0.00
	10 to 20	0.25	0.06	0.98	0.17	0.00
	Combined	0.23	0.00	0.85	0.23	0.00
Aerobic respiration rate measured on day five of incubation	0 to 10	0.62	0.04	0.92	0.43	0.00
	10 to 20	0.27	0.05	0.99	0.19	0.00
	Combined	0.61	0.00	0.91	0.62	0.00
Mean aerobic respiration rate	0 to 10	0.97	0.04	0.88	0.75	0.00
	10 to 20	0.26	0.05	0.99	0.19	0.00
	Combined	0.42	0.00	0.87	0.42	0.00
Respiratory ratio on day two	0 to 10	0.01	0.82	0.61	0.01	0.48
	10 to 20	0.01	0.67	0.74	0.00	0.21
	Combined	0.00	0.61	0.42	0.00	0.17
Respiratory ratio on day five	0 to 10	0.96	0.06	0.97	0.75	0.00
	10 to 20	0.25	0.05	1.00	0.18	0.00
	Combined	0.38	0.00	0.98	0.40	0.00

Table A8 Probability results of Kruskal-Wallis tests with effects of amendments on loss of PHCs over field trial.

	Sampling depths (cm)	Probability *P*
% Loss of total extractable hydrocarbons (TEH) over trial	0 to 10	0.02
	10 to 20	0.01
	Combined	0.00
% Loss of fraction 2 petroleum hydrocarbons (PHC) over trial	0 to 10	0.01
	10 to 20	0.01
	Combined	0.00
% Loss of fraction 3 PHCs over trial	0 to 10	0.01
	10 to 20	0.01
	Combined	0.00
% Loss of fraction 4 PHCs over trial	0 to 10	0.05
	10 to 20	0.03
	Combined	0.00
Total loss of TEH over trial from contaminated soils	0 to 10	0.09
	10 to 20	0.08
	Combined	0.00
Total loss of fraction 2 PHCs over trial from contaminated soils	0 to 10	0.06
	10 to 20	0.05
	Combined	0.00
Total loss of F3 PHCs over trial from contaminated soils	0 to 10	0.02
	10 to 20	0.02
	Combined	0.00

Table A8 continued

	Sampling depths (cm)	Probability P
Total loss of fraction 4 PHCs over trial from contaminated soils	0 to 10	0.03
	10 to 20	0.03
	Combined	0.00
Total loss of fraction 3 PHCs over trial from non-contaminated soils	0 to 10	0.58
	10 to 20	0.40
	Combined	0.21
Total loss of fraction 4 PHCs over trial from non-contaminated soils	0 to 10	0.01
	10 to 20	0.00
	Combined	0.00

Table A9 Probability results of non-parametric ANOVAs with effects of crude oil contamination and amendment on soil properties and vegetation invasion at end of experiment

		Scheirer-Ray-Hare test			Kruskal Wallis test	
	Sampling depths, cm	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Soil moisture content (mass basis) at end of trial	0 to 10	0.60	0.32	0.40	0.60	0.01
	10 to 20	0.65	0.37	0.52	0.65	0.02
	Combined	0.96	0.08	0.19	0.96	0.00
Soil bulk density at end of trial	N/A	0.18	0.14	0.91	0.31	0.01
Total vegetation cover at end	N/A	0.05	0.15	0.70	0.01	0.00
Root biomass at end of trial	N/A	0.09	0.15	0.76	0.02	0.00

Table A9 continued

		Scheirer-Ray-Hare test			Kruskal Wallis test	
	Sampling depths, cm	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Soil pH at end of trial	0 to 10	0.46	0.03	0.97	0.46	0.00
	10 to 20	0.70	0.03	0.98	0.70	0.00
	Combined	0.87	0.00	0.97	0.87	0.00
Soil total organic carbon at end of trial	0 to 10	0.91	0.05	0.99	0.91	0.00
	10 to 20	0.87	0.05	0.88	0.86	0.00
	Combined	0.84	0.00	0.93	0.84	0.00
Base saturation of soil at end of trial	0 to 10	0.81	0.04	0.93	0.81	0.00
	10 to 20	0.53	0.05	0.91	0.53	0.00
	Combined	0.61	0.00	0.85	0.61	0.00
Soil calcium content at end of trial	0 to 10	0.68	0.03	1.00	0.68	0.00
	10 to 20	0.50	0.04	0.96	0.50	0.00
	Combined	0.93	0.00	0.99	0.92	0.00
Soil calcium content at end of trial	0 to 10	0.68	0.03	1.00	0.68	0.00
	10 to 20	0.50	0.04	0.96	0.50	0.00
	Combined	0.93	0.00	0.99	0.92	0.00
Soil potassium content at end of trial	0 to 10	0.34	0.03	0.99	0.34	0.00
	10 to 20	0.53	0.03	0.99	0.53	0.00
	Combined	0.38	0.00	0.99	0.38	0.00

Table A9 continued

	Sampling depths, cm	Scheirer-Ray-Hare test Effect of fuel	Scheirer-Ray-Hare test Effect of amendments	Scheirer-Ray-Hare test Fuel x amendment interaction	Kruskal Wallis test Effect of fuel	Kruskal Wallis test Effect of amendments
Soil magnesium content at end of trial	0 to 10	0.72	0.03	1.00	0.72	0.00
	10 to 20	0.58	0.04	0.94	0.58	0.00
	Combined	0.96	0.00	1.00	0.96	0.00
Soil sodium content at end of trial	0 to 10	0.55	0.04	0.96	0.55	0.00
	10 to 20	0.88	0.05	0.92	0.88	0.00
	Combined	0.41	0.00	0.91	0.41	0.00
Soil electrical conductivity at end of trial	0 to 10	0.71	0.05	0.99	0.70	0.00
	10 to 20	0.18	0.06	0.97	0.18	0.00
	Combined	0.42	0.01	1.00	0.42	0.00
Soil sodium adsorption ratio at end of trial	0 to 10	0.81	0.02	0.99	0.81	0.00
	10 to 20	0.82	0.03	0.93	0.82	0.00
	Combined	0.82	0.00	0.96	0.82	0.00
Leachate production in 2003	N/A	0.00	0.70	0.48	0.00	0.56
Total leachate production over trial	N/A	0.00	0.51	0.27	0.00	0.33
Soil total nitrogen content at end of trial	0 to 10	0.19	0.05	1.00	0.19	0.00
	10 to 20	0.39	0.05	0.96	0.38	0.00
	Combined	0.11	0.00	0.97	0.10	0.00

Table A9 continued

			Scheirer-Ray-Hare test		Kruskal Wallis test	
	Sampling depths, cm	Effect of fuel	Effect of amendments	Fuel x amendment interaction	Effect of fuel	Effect of amendments
Soil ammonium content at end of trial	0 to 10	0.32	0.17	0.95	0.32	0.00
	10 to 20	0.75	0.11	0.94	0.75	0.00
	Combined	0.42	0.01	0.88	0.42	0.00
Soil carbon to nitrogen ratio at end of trial	0 to 10	0.01	0.45	0.97	0.01	0.05
	10 to 20	0.03	0.51	0.92	0.03	0.07
	Combined	0.00	0.18	0.90	0.00	0.00
Soil nitrate content at end of trial	0 to 10	0.05	0.11	0.78	0.05	0.00
	10 to 20	0.02	0.16	0.70	0.02	0.00
	Combined	0.00	0.01	0.59	0.00	0.00
Soil sulphate content at end of trial	0 to 10	0.85	0.04	0.98	0.85	0.00
	10 to 20	0.76	0.03	0.97	0.76	0.00
	Combined	0.88	0.00	1.00	0.88	0.00
Soil manganese content at end of trial	0 to 10	0.61	0.80	0.96	0.61	0.38
	10 to 20	0.38	0.53	0.88	0.38	0.09
	Combined	0.33	0.41	0.95	0.33	0.04

Dilution Correction for Microbial Population Estimates (Zuberer 1994)

Example: 10 g of moist soil was added to 90 mL of dilution solution to create a ten-fold soil dilution. The oven-dry moisture content of the soil was later determined to be 33%.

$$\text{Actual dilution} = \frac{\text{\# g moist soil + g of dilution solution (assumed 1 mL = 1g)}}{\text{\# g moist soil x (1 – fraction of water in moist soil)}}$$

$$\text{Actual dilution} = \frac{\text{10 g moist soil + 90 g dilution solution}}{\text{10 g moist soil x (1 – 0.33)}} = 15$$

The soil dilution is thus 1:15, instead of 1:10 as intended. The population estimate from plate counting was thus corrected by multiplying the colonies counted by 1.5:

$$\text{Multiplication factor for plate counts} = \frac{\text{actual dilution}}{\text{intended dilution}} = \frac{15}{10} = 1.5$$

Media for Enumeration of Total Aerobic Heterotrophs

This procedure is designed to yield bacteria and, if present, actinomycetes (Walker and Colwell 1976). Difco plate count agar, a commercial nutrient-enriched medium containing 1.5% agar, was purchased from Fisher Scientific. The medium was prepared as directed, with 23.5 g of granulated plate count agar (PCA) dissolved per liter of distilled water, using 4, 6 and 12 L Erlenmeyer flasks filled to half-capacity. Availability of flasks and autoclave space allowed 16 to 20 L of medium to be prepared in one batch. The medium was autoclaved in the flasks for 35 minutes at 121 °C and 124 kPa. After cooling to 56 °C in a water bath, approximately 25 mL of medium was poured into each Petri dish using a New Brunswick MP-320 Pourmatic Automatic Petri Dish Filler (Zuberer 1994; Jobson 2004).

Media for Enumeration of Total Viable Fungi

Soil plating does not distinguish between fungal spores or hyphae (Parkinson 1994). Martin's medium was prepared using the ingredients listed below. This procedure prepares 1 L of media, but 4, 6 and 12 L Erlenmeyer flasks filled to half-capacity were used to prepare 16 to 20 L of medium in one batch. Rose Bengal solution was prepared by dissolving 1 g of Rose Bengal in 100 mL deionised water. The ingredients are 10.0 g glucose, 5.0 g peptone, 0.50 g K_2HPO_4, 0.50 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 3.3 mL Rose Bengal solution and 15.0 g granulated agar. The medium was autoclaved in the flasks for 35 minutes at 121 °C and 124 kPa. After cooling to 56 °C in a water bath, 20 mg of filter-sterilised streptomycin sulphate was added with a syringe. Approximately 25 mL of medium was then poured into each Petri dish using a New Brunswick MP-320 Pourmatic Automatic Petri Dish Filler (Parkinson 1994; RTDF 1999).

Media for Enumeration of Hydrocarbon-Degrading Microorganisms

Each well in sterile disposable 96-well microtiter plates was filled with 180 μL of sterilised Bushnell-Haas medium supplemented with 2% NaCl (Haines et al. 1996; Wrenn and Venosa 1996; Nichols et al. 1997; Eriksson et al. 2000). Reagents to prepare 1 L of Bushnell-Haas medium are 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g calcium chloride ($CaCl_2$), 0.1 g iron (III) chloride ($FeCl_3$), 1.0 g K_2HPO_4, 1.0 g KH_2PO_4 and 1.0 g NH_4NO_3.

Calculation of Microbial Biomass Carbon

The fraction of total biomass C extractable by K_2SO_4, designated K_{EC}, must be known to convert the mass of organic C extracted from soil to total soil microbial biomass C.

- mg organic C extracted = mg organic C extracted from fumigated soil – mg organic C extracted from non-fumigated soil – mg organic C in blank extractant
- mg soil microbial biomass C = mg organic C extracted / K_{EC}
- mg organic C in soil = DOC (mg dissolved organic C per L solution analysed) x dilution factor for filtrate x L soil solution where L soil solution = L K_2SO_4 extractant + L water in soil sample

Literature values of K_{EC} are approximately 0.35 (Horwath and Paul 1994; Martens 1995).

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